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16. ABSTRACT

The development of a rapid analytical method for determining chrysotile asbestos in water that requires substantially less time per analysis than electron microscopy methods is described. Based on the proposition that separation of chrysotile from other waterborne particulate would greatly simplify the task of detection, the research effort was directed toward establishing separation and concentration techniques. This investigation led to the development of a separation procedure whereby chrysotile is extracted from a water sample into an immiscible organic liquid phase. The procedure is called two-phase liquid separation (TPLS).

TPLS has been combined with a light microscopic intercept counting technique and with acolorimetric spot test detection technique to result in two complete rapid analytical methods. The TPLS-light microscopic (LM) method requires more expensive equipment than the TPLS-spot test method; however, the limit of detection of TPLS-LM method is 1.0 ng at the 99 percent confidence level, whereas the limit of detection of the TPLS-spot test method is approximately 100 ng. The TPLS-LM method, therefore, is recommended as a first choice, and the TPLS-spot test method is recommended for use under conditions that require no greater detection sensitivity than 100 ng per sample.

Experiments have shown that 75 percent of the chrysotile is extracted by TPLS.

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December 1978

DEVELOPMENT OF A RAPID ANALYTICAL METHOD FOR DETERMINING ASBESTOS IN WATER

by

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Analytical Chemistry Branch develops and assesses new techniques for identifying and measuring chemical constituents of water and soil.

The widespread use of asbestos-containing materials gives rise to concern about exposure of the general population to low level concentrations in air, water supplies, and food. Although hazards associated with the inhalation of asbestos at high concentrations are recognized, the health significance of ingested particles is not fully understood. Although electron microscopic methods are required for the quantitative determination of asbestos levels in water, such methods are expensive, time-consuming and require sophisticated equipment not usually available in a conventional chemical laboratory. There is a need, therefore, for a simple, rapid analytical method that can be used for the preliminary examination of a large number of water samples. This report describes the progress that has been made toward the development of a rapid analytical procedure for chrysotile asbestos.

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Director
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ABSTRACT

The development of a rapid analytical method for determining chrysotile asbestos in water that requires substantially less time per analysis than electron microscopy methods is described. Based on the proposition that separation of chrysotile from other waterborne particulate would greatly simplify the task of detection, the research effort was directed toward establishing separation and concentration techniques. This investigation led to the development of a separation procedure whereby chrysotile is extracted from a water sample into an immiscible organic liquid phase. The procedure is called two-phase liquid separation (TPLS).

TPLS has been combined with a light microscopic intercept counting technique, and also with a colorimetric spot test detection technique to result in two complete rapid analytical methods. The TPLS – light microscopic (LM) method requires more expensive equipment than the TPLS – spot test method; however, the limit of detection of TPLS-LM method is 1.0 ng, approximately 40,000 fibers, at the 99 percent confidence level, whereas the limit of detection of the TPLS – spot test method is approximately 100 ng or 4.0×10^6 fibers. The TPLS-LM method, therefore, is recommended as a first choice, and the TPLS – spot test method is recommended to be used under conditions that require no greater detection sensitivity than 100 ng per sample.

TPLS extracts chrysotile from water into isooctane after the chrysotile surface has been rendered hydrophobic by reaction with an anionic surfactant (dioctyl sodium sulfosuccinate). Extraction of the chrysotile from the water phase into the isooctane phase occurs as the two liquids are shaken in a separatory funnel. Agitation creates an emulsion that is broken by adding sodium chloride solution. The isooctane is then filtered to deposit the chrysotile on a filter where its concentration is analyzed by light microscopy or spot test procedures.

For light microscopic detection, the chrysotile is deposited on a Nuclepore filter, which has a relatively smooth surface. The filtered deposit is carbon coated to make it reflective and also to enhance the apparent fiber diameters to make more fibers detectable by light microscopy. Intercept counts are made of fiber segment lengths equal to the spacing between concentric circles in a calibrated eyepiece reticule. The measurement of total fiber length as the diameter of the filter is traversed is referred to a standard curve to obtain mass of chrysotile per filter deposit.

For spot test detection, the extracted chrysotile is deposited in a small (3-mm diameter) area on a Millipore filter. The chrysotile is reacted with manganous ion and washed, and the reacted manganous ion is detected by a spot test reaction between potassium periodate and tetrabase, which is catalyzed by manganese. Varying shades of blue serve to indicate different concentrations of chrysotile.

Experimental results have shown that 75 percent of the chrysotile is extracted by TPLS procedures. This is taken into account when evaluating analytical results.

This report was submitted in fulfillment of Contract No. 68-03-2199 by Battelle's Columbus Laboratories, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from June 1, 1975, to June 30, 1978, and work was completed. as of June 1978.

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SECTION 1

INTRODUCTION

The presence of asbestos in water supplies in the United States and Canada, as well as other parts of the world, has led to the need to rapidly analyze large numbers of natural- and industrial-water samples for asbestos content. Streams, runoff, lakes and city water systems are the major receptacles of asbestos contamination. Natural weathering of asbestos deposits, eroding of asbestos-cement city water pipes, and dumping of tailings from mining operations are some of the mechanisms by which water supplies become contaminated with asbestos. In the past, transmission electron microscopy (TEM) has been the primary method for determining asbestos concentrations, but the time and cost required to analyze a sample becomes prohibitive when dealing with a large number of sampling sites. Clearly, there is a need for a more rapid and less expensive analytical method.

The objective of the research program reported here was to develop a rapid analytical method for asbestos in water requiring substantially less time and cost and less sophisticated equipment than a method based on TEM. Research was performed to develop a rapid monitoring method that would eliminate from further examination those samples containing asbestos concentrations below significant levels. After screening, only those samples identified as having significant quantities of asbestos would be submitted for more definitive TEM analysis.

Asbestos analytical methodology employs TEM because the fiber sizes range well below the resolution capabilities of the light microscope, and health effects investigators require fiber-size-frequency data. This does not imply that the rapid method must obtain fiber sizes. It need only identify those samples that need to be fully characterized. Therefore, the detection method employed in the rapid procedure is based on bulk analysis.

The logic behind the approach taken in the development of a rapid method was guided by an awareness of problems arising from gross amounts of interfering particulate prevalent in most water samples. The sample could be rid of interfering organic matter by low-temperature ashing, but the inorganic particulate could not easily be eliminated to leave only the asbestos. Analysis either had to be carried out in the presence of both organic and inorganic particulate or in the presence of inorganic particulate or the asbestos had to be separated from the interfering particulate. Analysis could be performed for separated and concentrated asbestos with much less difficulty than for asbestos in the presence of interfering particulate. Accordingly, our research effort emphasized the development of a separation method for asbestos in order to facilitate its subsequent detection. The separation of chrysotile was investigated first because chrysotile is the most prevalent form of asbestos found in most sampling locations. Also, its surface chemistry is sufficiently different than most other waterborne particulate to make its selective separation possible.

The goal has been to develop a separation method that will not only eliminate interfering extraneous particulate, but will also reduce the time required for specimen preparation. Specimen preparation methods employed in conjunction with electron microscopy detection often require lengthy ashing periods to rid the sample of organic matter, but fail to eliminate masking inorganic particles from the ash residue. The developed separation method does not include an ashing step, but does selectively and rapidly separate and concentrate the asbestos directly from the original water sample.

Three approaches to chrysotile separation were explored. All exploited differences in electrical properties between chrysotile and other prevalent waterborne particles. The methods investigated were continuous particle electrophoresis (CPE), dielectric separation, and extraction of asbestos from aqueous suspension into an immiscible oil phase (two-phase liquid separation). Although CPE proved promising, there was greater success with the two-phase liquid extraction technique. Consequently, most of the research effort was directed toward the study of two-phase liquid separation (TPLS).

Attempts to apply TPLS to amphibole separation were not successful; however, this was a minor research effort. Early in the program it was decided to concentrate on chrysotile separation rather than concurrently try to devise a procedure for the separation of amphiboles. It is believed that the separation of amphiboles by modified TPLS procedures remains a possibility.

The complete analytical method for chrysotile includes TPLS coupled with light microscopic analysis of the chrysotile in the separated fraction. Alternatively, the separated and concentrated chrysotile is analyzed by a catalytic spot test procedure.

Two-phase liquid separation promotes the selective transfer of chrysotile from water to an immiscible organic liquid after the chrysotile surface has been rendered hydrophobic by reaction with an anionic surfactant. The organic liquid is then filtered to deposit the separated chrysotile on a Nuclepore filter. The filtered chrysotile is carbon coated and viewed with a light microscope employing bright field incident illumination at 500X, and counts are made of fiber segments as the diameter of the effective filter area is traversed. The counts thus obtained are translated into chrysotile mass (μg) per filter by means of a standard curve that was generated using standard filtered chrysotile suspensions.

The spot-test analytical procedure is applied to the TPLS fraction that has been deposited on an $\approx$3 mm diameter area on a Millipore filter. First, the deposited chrysotile is reacted with manganous ion and washed, and then the reacted manganese is detected by a chromogenic spot test reaction between potassium periodate and tetrabase, which is catalyzed by manganese.

The limit of detection of the TPLS – light microscopic method is 1 nanogram at the 99 percent confidence level. The smallest quantity of chrysotile detectable by the spot test is 100 nanograms. Although the spot test is much less sensitive, it can be used effectively in certain monitoring situations. The relationship between mass and total number of chrysotile fibers is variable and strongly dependent upon fiber dimensions. All water samples show a broad range of fiber size distribution; for simplification in this work it has been assumed that the average fiber is 1 μm long and 0.1 μm wide. When this assumption is made 1 ng is equivalent to approximately 40,000 fibers.

SECTION 2

CONCLUSIONS

(1) Continuous particle electrophoresis (CPE) will separate chrysotile, but CPE will not handle sufficiently large volumes of particulate to be used in an analytical method.

(2) Dielectric separation as investigated is ineffective for chrysotile.

(3) Two-phase liquid separation of chrysotile from water samples coupled with light microscopic analysis of the separated chrysotile by intercept counting will detect chrysotile quantities as low as 1.0 ng at a 99 percent confidence level. Assuming an average chrysotile fiber size of 1 μm length and 0.1 μm width, 1 ng is the equivalent to approximately 40,000 fibers.

(4) TPLS extracts 75 percent of those chrysotile particle sizes detectable by light microscopy.

(5) The sensitivity of the light microscopic detection procedure is high over the concentration range above 1.0 ng; an average of nine counts is registered at 1.0 ng and 15,000 counts at 1000 ng.

(6) Few interferences occur in TPLS; it is surprisingly selective. Organic filaments and platy mineral particles, however, are occasionally extracted along with chrysotile. The filaments can be distinguished from chrysotile with a little practice. The presence of the platy mineral can result in some of the chrysotile being obscured from view.

(7) The TPLS – spot test procedure requires relatively inexpensive equipment and can be run with a minimum of training by an individual with no specific background.

(8) The detection limit of the spot test procedure is approximately 100 ng.

(9) The spot test is selective for chrysotile; no interferences were encountered while running real water samples.

(10) TPLS can separate as large as 0.4-mg quantities of chrysotile from 100-ml water aliquots.

(11) Degree of agitation, pH, concentration of surfactant, the ratio of the volumes of oil/water, and the number of extractions from a single water aliquot are the most critical TPLS factors.

(12) Percent chrysotile recovery by TPLS is optimized:

- at pH 3.5 to 5.0
- when the oil/water volume ratio is 1:2
- when shaking is the mode of agitation
- when 2.5×10^{-3} M anionic surfactant concentration is used
- when five extractions are made from the same water aliquot.

(13) TPLS favors the extraction of longer fibers with larger aspect ratios.

(14) Filtration and resuspension is a practical means of concentrating particulate from large water volumes for TPLS.

(15) It is not as feasible to scale up TPLS to handle large water volumes as it is to filter and resuspend particulate in smaller, more manageable water volumes for TPLS.

(16) The results of the interlaboratory analyses at Battelle and ORF were in agreement within a factor of 4, except on Sample No. 4 in which Battelle found only 1/10 of the chrysotile reported by ORF. Battelle's TPLS – light microscopic result in this case agreed with the electron microscopic result obtained at ORF.

(17) Approximately eight samples can be prepared and analyzed by the TPLS – light microscopic method by two people in one day.

SECTION 3

RECOMMENDATIONS

As with any new analytical technique, one must proceed with caution and become thoroughly familiar with the application of two-phase liquid separation, light microscopic detection and spot test application before relying on the analytical results. It is recommended, therefore, that the results of these procedures be compared initially to results obtained on the same samples by electron microscopy. Afterwards, when there is sufficient confidence in the method's effectiveness, one can progress toward the application of TPLS coupled with either of the detection procedures as requirements dictate without comparison to electron microscopic analytical results.

Although water samples were analyzed using both TPLS coupled with light microscopic counting and the TPLS – spot test procedure, it was not possible to investigate all varieties of water samples. Thus, unanticipated interferences may occur to give high or low results under sample conditions not investigated.

It is recommended that the developed methodology be applied first to monitoring variations in chrysotile concentrations from well characterized sources, and then, as experience develops, be applied to the analysis of chrysotile from different sources.

SECTION 4

EXPERIMENTAL WORK

This section of the report discusses the experimental work performed to develop separation and detection methods for chrysotile. Separation experiments explored dielectric separation, continuous particle electrophoresis, and two-phase liquid separation. When promising results were obtained using TPLS to separate chrysotile, emphasis was placed on the optimization of this process. No further search was made for other separation methods for chrysotile. Also, when initial experiments to separate amphiboles by various modifications of TPLS were not completely successful, it was decided to concentrate the entire effort on improvement of the separation of chrysotile.

Several detection methods were investigated with the objective of developing a method requiring less time and less expensive equipment than electron microscopy. Spot-test reactions, changes in flow rate through filtered chrysotile deposits, X-ray diffraction, and light microscopy were evaluated. Light microscopy of the separated and filtered chrysotile was the most successful detection method found, and it has been written into the detailed instructions for the final method. A spot-test procedure was also developed for chrysotile detection and is recommended as an alternative.

PHASE I: SEPARATION EXPERIMENTS

Initial Separation Experiments

Continuous Particle Electrophoresis –

The Beckman Continuous Particle Electrophoresis (CPE) System was used in experiments designed to separate and concentrate chrysotile from synthetic mixtures of chrysotile and diatomaceous earth. The operating principle of CPE and related experiments are described in the following sections.

Operating Principle of CPE –

Simply stated, the operating principle of Beckman CPE is as follows. A suspension of sample material is introduced as a fine, steady stream into a vertical, flowing "curtain" of electrolyte. A horizontal dc electrical field is applied to the curtain. Each particle has two components of motion: a vertical component (V_v) that is the same as the curtain velocity, and a horizontal component (V_h) that is proportional to the electrophoretic mobility or zeta potential of the particle. Each different particulate component is therefore deviated a different angle, α, from the vertical, given by

$$\tan \alpha = \frac{V_h}{V_v}$$

The net result at the lower end of the cell is a particle band position that is proportional to the zeta potential of the particles. Where a mixture of particles having different electrophoretic mobilities is analyzed, each type of particle will assume a different band position.

The possibility of fractionating different types of particles by CPE assumes a certain minimum difference in their electrophoretic mobilities. The relative mobilities in a mixture can frequently be manipulated. One useful procedure is to change the pH. Others include the use of polyvalent cations, and use of surfactants and various agents that alter the surface characteristics of the particles.

The CPE instrument is shown in Figure 1. This system can be separated into five subsections: (1) curtain flow system, (2) sample flow system, (3) electrode rinse systems, (4) cooling system, and (5) power supply. The curtain flow system provides a filtered source of the electrolyte at a constant flow rate to the cell. The sample flow system provides a means of continuously metering a sample into the flowing curtain medium. The electrode rinse system is designed to remove electrolysis products from the electrodes and thereby maintain a high degree of pH stability within the curtain. The power supply is designed to provide a constant voltage gradient within the cell regardless of changes in internal impedance or electrolyte conductivity.

The electrodes, located in two chambers positioned at the sides of the curtain cell, are isolated from the curtain by semipermeable membranes. This separation prevents mixing of the electrode rinse with the curtain buffer. The sample is introduced at the top of the curtain and is thereby diluted by the curtain buffer solution as it flows downward between the electrodes. The sample particles are influenced by the voltage gradient to migrate laterally, and finally to be discharged through drainage tubes positioned at uniform (1 mm) intervals across the bottom of the curtain cell. The effluent is thus divided into 48 fractions and collected in test tubes.

According to the instructions in the use of the CPE system, most particles possess a higher zeta potential at higher pH conditions. Consequently, they recommend the utilization of buffer media at relatively high pH values. The instructions also suggest the use of a 30 V/cm voltage gradient for most applications. Therefore, most of the initial CPE experiments were carried out at pH values greater than 7.0 – employing a voltage gradient of 30 V/cm.

CPE Experiments –

CPE experiments were conducted at pH 8.6, 3.6, and 10.7. Runs were made at pH 8.6 and 10.7 because the CPE instructions stated that better separation usually was effected at higher pH conditions. The run at pH 3.6 was made because literature[1] indicated that the greatest positive surface charge was developed on chrysotile at this pH.

The first attempt to separate chrysotile from diatomaceous earth was made in a veronal buffer at pH 8.6. An initial run was made with pure chrysotile dispersed in filtered, deionized water containing 1.9% aerosol OT to determine into which tubes the chrysotile would be discharged. It was found that the bulk of the chrysotile was collected in the test tubes numbered 20 through 29. This was determined by filtering 5-ml aliquots from each tube onto 25-mm HAWP Millipore filters and examining prepared specimens by electron microscopy. The specimens were prepared by cutting a piece from the center of the filter and dissolving the filter material away in an acetone bath while the deposit side was in contact with a carbon support

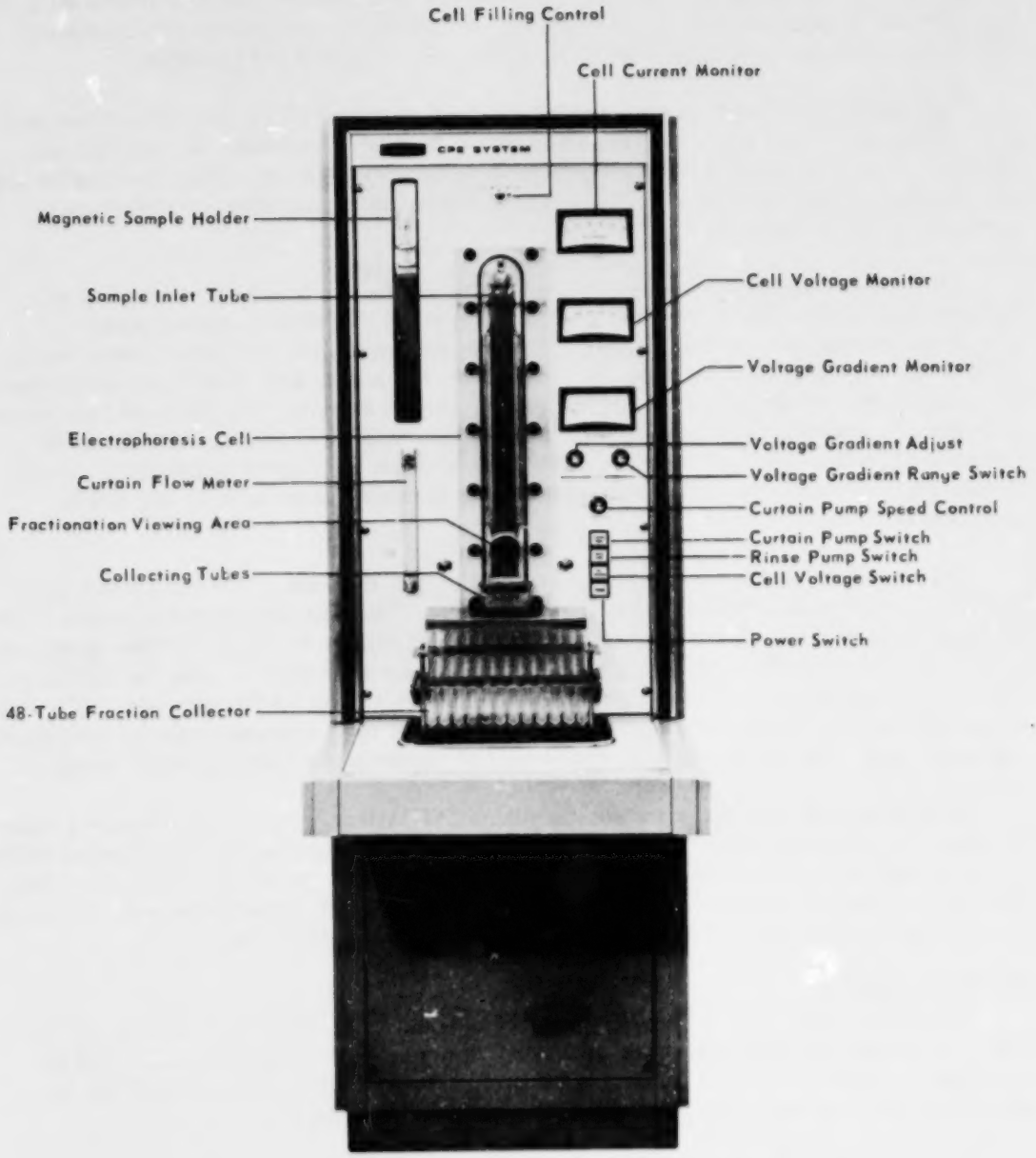

Figure 1. CPE instrument.

film held by a mesh electron microscope grid. Electron micrographs were taken of preparations containing asbestos, and those micrographs showing the bulk of the asbestos were contact printed. The resulting contact print is included in Figure 2.

A second run at pH 8.6 was made on pure diatomaceous earth also dispersed in filtered deionized water containing 1.9% aerosol OT. The diatoms were discharged in the same tubes as was the chrysotile; consequently, no further experiments were performed at pH 8.6.

Subsequently, separations were attempted at pH 3.6 (phosphate buffer) at which agglomeration of particulate resulted in plugging of the tubes through which the effluent from the curtain cell was discharged. The addition of the dispersant Aerosol OT did not prevent agglomeration at pH 3.6.

Next, runs were made at pH 10.7 (in 0.001 M Na_2CO_3) first separately on pure chrysotile and pure diatomaceous earth dispersed in 1.9% Aerosol OT in deionized water. These experiments indicated that separation might be obtained at this pH; the chrysotile was concentrated primarily in tubes 20 through 24 and diatoms in tubes 25 through 30. Then, a run was made in which separation was attempted on a 10:1 by weight (7.0 mg and 0.7 mg in 100 ml H_2O) mixture of diatomaceous earth and chrysotile, respectively. This mixture also was dispersed in 1.0% Aerosol OT in filtered deionized water. As predicted by the runs on the pure materials, a separation was effected. Figure 3 shows electron microscope preparations from test tubes numbered 20 through 27. Although some diatoms were found in tubes 22, 23, and 24, the degree of separation represents a marked improvement over the original mixture shown in Figure 4.

Possibly, deviation from the 30-V/cm voltage gradient would yield even better separations. Also, instrument instructions state that lower curtain flow rates result in better separations. All experiments were conducted at a curtain flow rate of 20 ml/minute with a sample introduction rate of 50 µl/minute. Of course, as the curtain flow and sample introduction rates are decreased, longer times are required for CPE separations. Compromises must be made in achieving the desired balance between degrees of CPE separation and run duration.

At this point, concurrent experiments with two-phase liquid separation proved more successful, and CPE studies were discontinued.

Dielectric Separation

Dielectric separation[2] is based on the relationship between the dielectric constants of suspended particulate material and that of the suspending liquid. When the suspending liquid has a lower dielectric constant than the particle, the particle is attracted to immersed electrodes when either an ac or dc voltage is imposed. Theoretically, when the difference is the least between the dielectric constants of the liquid and the particle type to be separated, the most effective separations are obtained.

First experiments were performed on pure chrysotile suspended in liquids having relatively low dielectric constants compared to chrysotile (dielectric constant = 33.7) in order to study the attraction of chrysotile to the electrodes at various voltages under both ac and dc conditions. Most of these trials were carried out in Freon MF having a dielectric constant of 2.4. It was

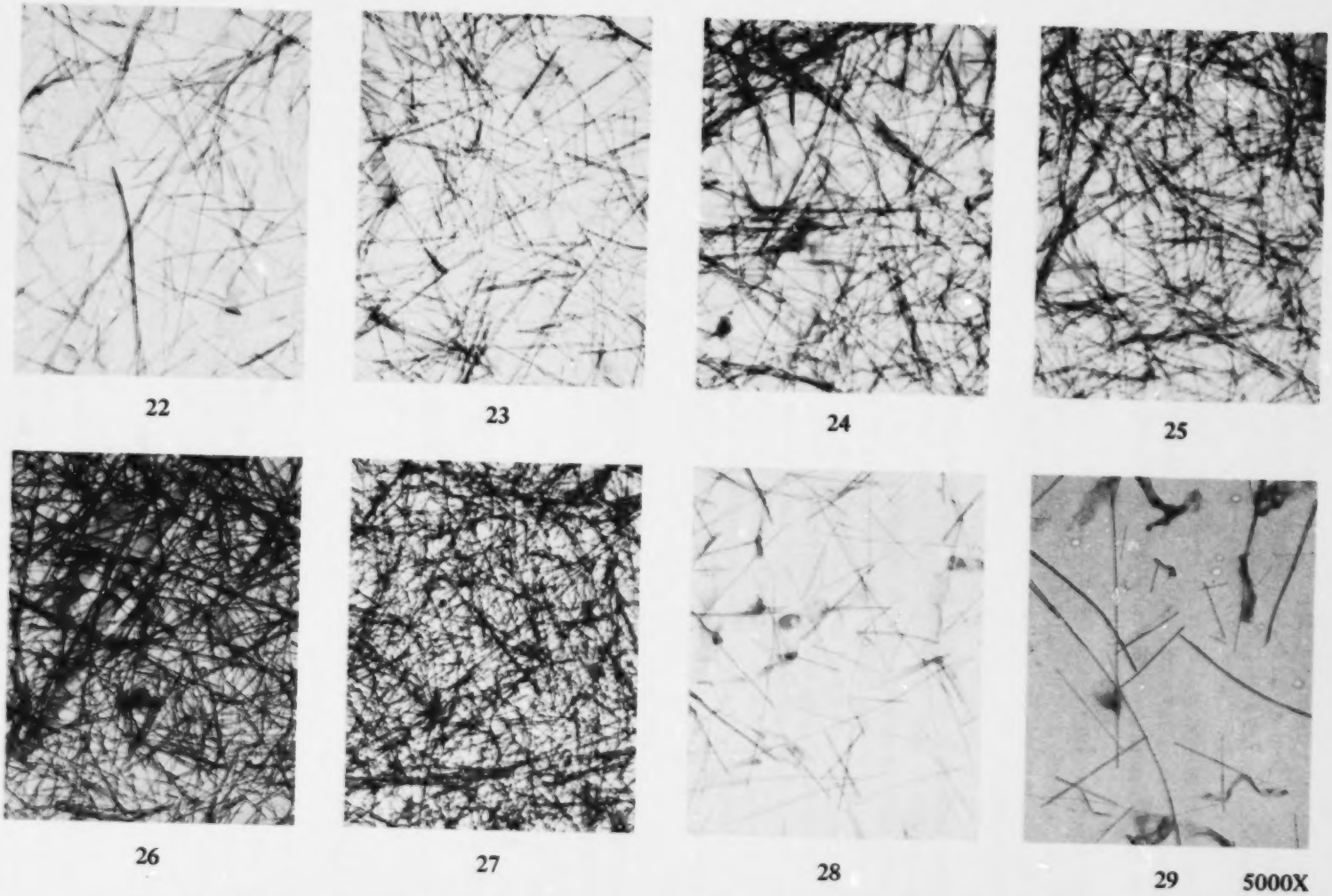

Figure 2. Electron micrographs of effluents collected in test tubes 22 through 29 in a CPE run made at pH 8.6 showing how pure chrysotile is confined to a band position in the curtain cell.

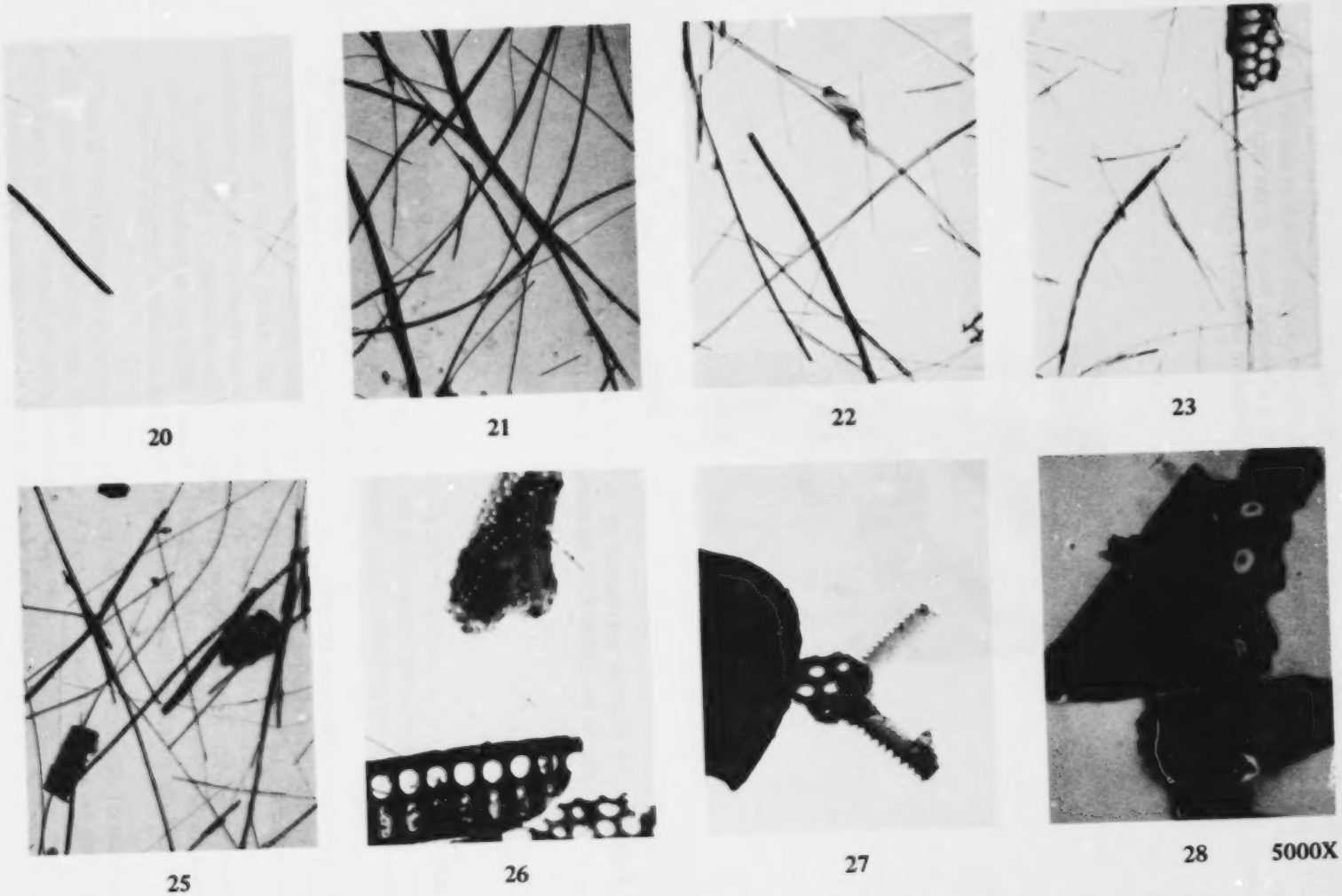

Figure 3. Electron micrographs of effluents collected in CPE test tubes 20 through 28 showing separation of diatoms from chrysotile in a run at pH 10.7.

found that chrysotile was best attracted and held at 200 Vdc to electrodes separated by a distance of approximately 1 cm. Below 200 V the chrysotile did not collect as rapidly on the electrodes, and above 200 V a turbulence developed that dislodged much of the deposit. Consequently, 200 Vdc was employed in subsequent dielectric separation experiments.

5000X

Figure 4. An electron micrograph of the original mixture of diatoms and chrysotile before CPE separation at pH 10.7.

Although chrysotile was deposited on the electrodes, it was not held well enough to lift the electrodes from the liquid and thereby remove the deposit. In order to be able to retain the chrysotile deposit on the electrodes while undeposited particulate was removed, a flow-through cell was designed. The cell design permitted gentle flushing of the liquid containing undeposited suspended particulate from the cell without disturbing the chrysotile deposit. A reservoir that contained particle-free liquid and that had a drain on the side delivered this liquid to the dielectric cell positioned below. The dielectric cell, which held 40 ml, also had a drain at the bottom which could be adjusted to a flow which matched the rate of introduction of the particle-free liquid. After the deposit had formed on the electrodes, the unattached particulate was flushed from the cell with 400 ml of liquid. Then the deposited particulate was resuspended in the remaining liquid and drained from the cell into a separate container.

Investigations of dielectric separation were carried out primarily in methyl, ethyl, and isopropyl alcohols because their dielectric constants are below that of chrysotile but relatively high compared to most other liquids. Also their dielectric constants can be altered upward with the addition of water to create solutions that are even nearer to the dielectric constant of

chrysotile. For example, water has a dielectric constant of 80 and ethyl alcohol a constant of 24. The change in dielectric constant is a straight line function, and thus 90% ethyl alcohol-10% water solution has a dielectric constant of 30. Separation trials were made, therefore, first with pure ethyl, methyl, and isopropyl alcohols and later with ethyl alcohol to which 10% water was added to elevate the dielectric constant.

First, separate suspensions of pure chrysotile and pure diatomaceous earth were run in various candidate liquids. In cases where it was observed that chrysotile was deposited and diatomaceous earth was not, 10:1 mixtures by weight of diatomaceous earth and chrysotile were run. Figure 5 illustrates how chrysotile was deposited on the anode from an isopropyl alcohol suspension of chrysotile. Despite the fact that this was originally a suspension of chrysotile fibrils in the colloidal size range, a visible deposit was formed on the electrode.

Always before making a run, the particulate was dispersed ultrasonically in the dielectric liquid without the aid of additives. On the basis of previously described experiments, pure methyl alcohol, pure isopropyl alcohol, and 90% ethyl alcohol-10% water solution were used in dielectric separation experiments. Although in each case some enrichment of asbestos was realized, the results were inferior to continuous particle electrophoresis and two-phase liquid separation. It appears that interaction may occur between chrysotile and diatoms to result in the attraction of both species to the electrode. Also, inevitably, as asbestos particles agglomerated, a mass was formed that trapped diatoms as they circulated in the dielectric liquid.

Experimentation with dielectric separation was curtailed in favor of the pursuit of the more promising two-phase liquid separation.

Two-Phase Liquid Separation

Two-phase liquid separation was conceived as a possible means of chrysotile separation. First experiments were successful, but success occurred somewhat accidentally. The following section discusses the process of TPLS discovery.

Evolution of TPLS for Chrysotile

The first TPLS experiment was conducted in an attempt to separate chrysotile from a 10:1 by weight synthetic mixture of diatomaceous earth and chrysotile that had been used in CPE experiments. The original mixture contained 7.0 mg of diatomaceous earth and 0.7 mg of chrysotile dispersed ultrasonically in 100 ml of 0.001 N Na_2CO_3 containing 0.1% Aerosol OT. The mixture had been treated ultrasonically to create a dispersion that would not clog the CPE discharge tubes.

Three ml of the original synthetic mixture was diluted by the addition of 10 ml of 0.001 N sodium carbonate, combined with 50 ml of benzene containing 0.5 ml of oleic acid, and agitated ultrasonically in a separatory funnel. The oleic acid addition was made because it was stated[1] that long-chain aliphatic acids are chemisorbed by chrysotile. It was reasoned that chemisorption of oleic acid would create a hydrophobic surface on the chrysotile fiber and thus promote its transfer to the benzene phase.

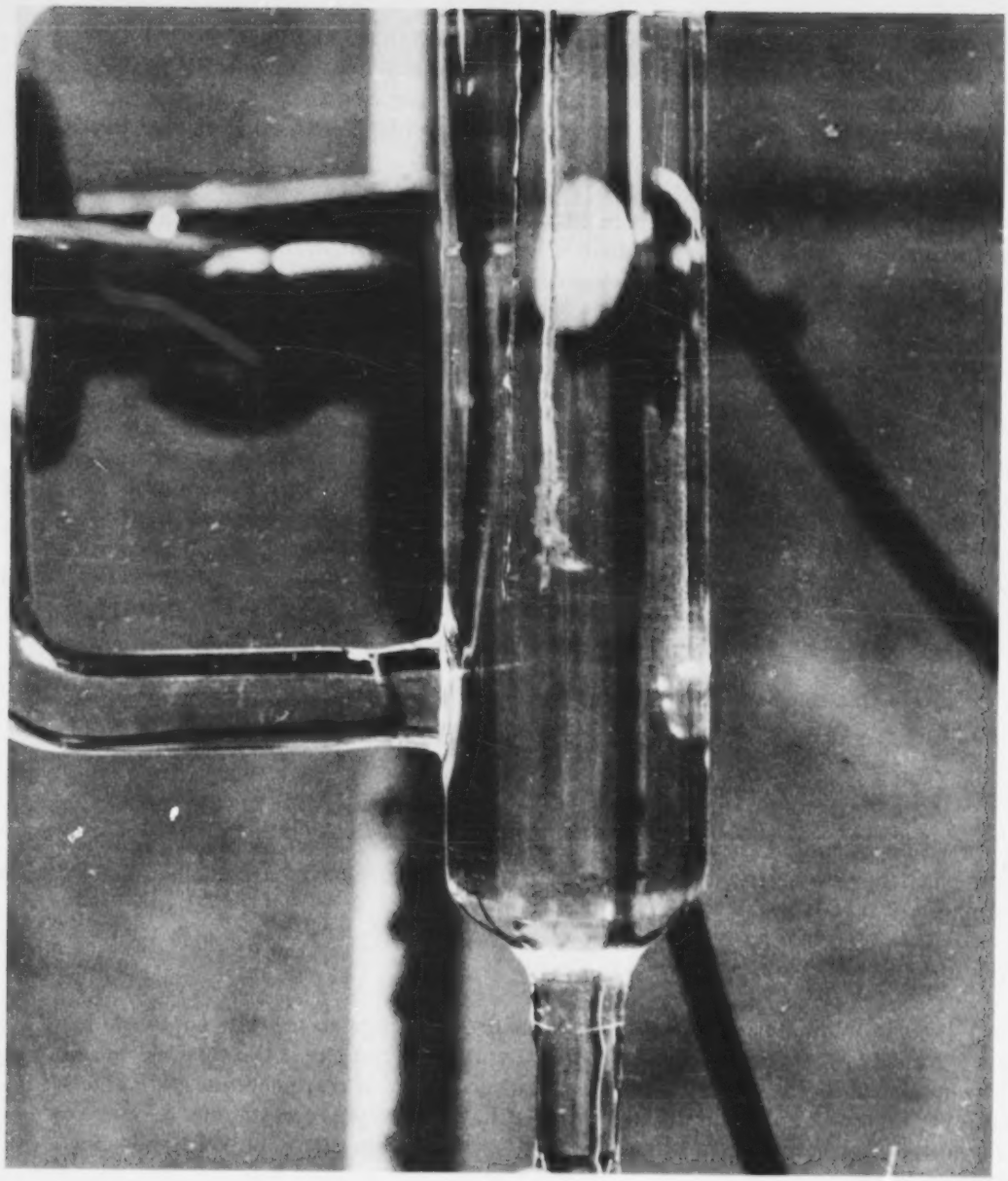

Figure 5. Chrysotile collected on the anode in the dielectric cell containing isopropyl alcohol.

An emulsion formed upon agitation separated into a benzene layer, a cream layer, and a water layer after standing overnight. One ml of the benzene layer was pipetted off and filtered through a 25-mm Millipore filter having a 0.45 μm pore size. Ten drops of the water layer were also filtered. Both filtered residues were prepared for electron microscopy. When the preparations were examined in the electron microscope it was found that the particulate in the benzene phase was comprised almost entirely of chrysotile (Figure 6) and that the particulate in the water phase was primarily diatomaceous earth (Figure 7).

These experimental results set the stage for further experiments designed to determine the critical factors involved in TPLS.

Results of the first experiment indicated that the oleic acid addition might be effecting a separation as predicted. In additional experiments employing comparable synthetic mixtures of 10:1 by weight of diatoms and chrysotile suspended in water containing no sodium carbonate or Aerosol OT, however, the addition of oleic acid did not promote chrysotile separation. Then, 0.05% of Aerosol OT was added to the water suspension, and another trial was made in which 50 ml of the water suspension was shaken in a separatory funnel with 50 ml of benzene containing 0.5 ml of oleic acid; again chrysotile was found to be selectively transferred to the benzene phase. Subsequent experiments in which the oleic acid was eliminated, but in which Aerosol OT was added, effected chrysotile separation. Other experiments without Aerosol OT or any other additions resulted in no chrysotile separation. It was concluded, therefore, that Aerosol OT was reacting with the surface of the chrysotile to promote its transfer to the benzene phase.

This discovery led to the investigation of TPLS mechanisms and conditions that affect percent recovery and selectivity. The early experiments were carried out with benzene; however, because of the possible toxicity of benzene, isooctane was ultimately used as the oil phase. The following section discusses the mechanisms identified through pertinent literature and experimentation.

The Mechanisms of TPLS –

Essentially, two-phase liquid separation selectively extracts chrysotile from a water sample into a water-immiscible oil phase, such as benzene, mineral spirits, or isooctane, after the surface of chrysotile fibers is made hydrophobic through a reaction with an anionic surfactant. TPLS selectivity is based on the difference between the zeta potential of chrysotile and the zeta potentials of most other waterborne particulate. The surface charge of chrysotile is positive over a pH range from 2 to 11.4; whereas most other waterborne particulate is negative over this pH range. The chrysotile, therefore, selectively reacts with the negatively charged anionic surfactant, becomes hydrophobic, and is transferred from the water phase to the oil phase.

Speil and Leinweber[1] state that chrysotile reacts with certain anionic wetting agents, such as Aerosol OT, and that this reaction is accompanied by a strong chemisorption of the agents with permanent modification of the surface. Because MgOH groups of the brucite layer are presented for reaction on the surface of chrysotile[3], the Aerosol OT (dioctyl sodium sulfosuccinate) most likely reacts with the brucite layer to create a tightly bound organic coating covering the fiber surface.

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Figure 6. Chrysotile separated from diatomaceous earth in the first TPLS experiment.

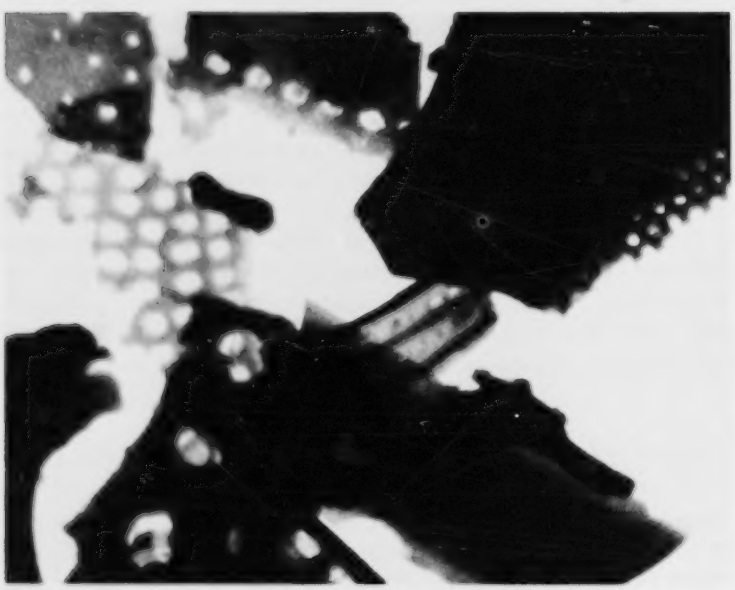

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Figure 7. Particulate remaining in the water phase in the first TPLS experiment.

Reinders[4] has defined interfacial tension conditions governing transfer of dispersed solids from the aqueous phase to the oil phase. If γ_{wo} is the interfacial tension of the oil-water interface, γ_{sw} is the interfacial tension of the solid-water interface, and γ_{so} is the interfacial tension of the solid-oil interface, the following conditions may exist.

(1) If $\gamma_{so} > \gamma_{wo} + \gamma_{sw}$, the solid remains suspended in the water phase.

(2) If $\gamma_{sw} > \gamma_{wo} + \gamma_{so}$, the solid is transferred to the oil phase.

(3) If $\gamma_{wo} > \gamma_{so} + \gamma_{sw}$, or if none of the interfacial tensions is greater than the sum of the other two, the solid will concentrate at the oil-water interface.

Condition (1) exists when the solid surface is hydrophilic and condition (2) or (3) may exist when the solid surface is hydrophobic. Experimentally, it can be determined which condition prevails by contact angle measurement.

No contact angle measurements have been made during the investigation of the chrysotile separation. They are rather difficult to make on a fibrous mineral because the surface cannot be polished smooth.

The sign and magnitude of the zeta potential of the mineral surface is affected by the pH. In turn, the zeta potential determines whether and to what degree a reagent will react with the mineral surface.

In the case of stoichiometric chrysotile, the zeta potential is positive in the pH range below the isoelectric point (zero point charge), which occurs at pH 11.4, attaining a maximum positive value in the range pH 3 to pH 4. Above pH 11.4 the zeta potential of chrysotile is negative.

Having a positive zeta potential at pH 7.0 and below is relatively unique among waterborne particles. This phenomenon provides the basis for separation of chrysotile from other particles. By making its surface hydrophobic through reaction with anionic reagents at <pH 11.4, the condition $\gamma_{sw} > \gamma_{wo} + \gamma_{so}$ is created to promote selective transfer of chrysotile to the oil phase. Aerosol OT, MT-70, and MO-70 surfactants, which are anionic analogous alkyl sodium sulfosuccinates, have been employed successfully in the separation of chrysotile.

The idealized formula for stoichiometric chrysotile[5] is $Mg_3Si_2O_5(OH)_4$. The fibrils have a rolled sheet structure composed of an outer layer of magnesia bonded to a layer of silica. Hydrated chrysotile presents MgOH groups for reaction with reagents. Chowdhury and Kitchener state that the higher the magnesia content the greater is the positive zeta potential. Also, there is evidence that a greater degree of hydration of the magnesia surface increases electrophoretic mobility. When all magnesia is leached out under acid conditions or by weathering, a silica gel pseudomorph that is left has a strongly negative zeta potential. This structure, not meeting the compositional criteria, is not chrysotile.

According to Lai and Fuerstenau[6], anionic reagent (collector) adsorption by or reaction with the mineral surface is primarily determined by the reagent concentration and the pH as it affects zeta potential. When reagent concentration and mineral zeta potential are optimum, the adsorption density of the reagent, as expressed in moles/cm^2, on the mineral surface is at a

maximum. As a result, the hydrophobicity also becomes the highest possible with that particular reagent.

The degree of hydrophobicity is also a function of reagent type. Those reagents having larger hydrophobic alkyl groups render the mineral surface more hydrophobic. Lai and Fuerstenau also found that the percent recovery of alumina from water suspension into isooctane, using sodium akyl sulfonate reagents with C8, C10, C12, and C14 alkyl groups, increased markedly with larger carbon chain length.

Heeding this finding, a search was made for a sodium sulfosuccinate having larger alkyl groups than the octyl groups of Aerosol OT. A reagent was found, Monawet 70 (MT 70), which is ditridecyl sodium sulfosuccinate. Experiments with this compound established that it also promoted transfer of chrysotile to the oil phase. Subsequent experiments (to be discussed later) indicated improved recovery but also reduced selectivity when compared to dioctyl sodium sulfosuccinate (MO-70 and Aerosol OT).

Most of the literature discusses adsorption of reagents. The reaction of the sulfosuccinates with the chrysotile surface may be more than adsorption, however: Speil and Leinweber[1] state that the highly reactive chrysotile surfaces causes many interesting surface reactions to take place that are intermediate between simple adsorption and true chemical reaction. They also report that the reaction of chrysotile with certain anionic wetting agents, such as Aerosol OT, is accompanied by a strong chemisorption of the reagents with permanent modification of the surface.

In TPLS, an organic liquid that is immiscible with water is agitated with water, and the transfer of suspended chrysotile from the water phase to the oil phase is promoted by the anionic surface active agent. The degree of interpenetration of phases as it affects the total interfacial contact is of critical importance in recovery of particles. Interfacial contact is determined by the intensity and mode of agitation, the oil/water volume ratio, and the surfactant concentration that controls emulsion formation.

Continual agitation is more effective than intermittent agitation because the droplets are not allowed time to form a stable emulsion. Consequently, the total interfacial contact becomes a function of agitation time and intensity.

The anionic reagent concentration must be high enough to lower the γ_{wo} to the point that contact between the phases is optimized. Lai and Fuerstenau found that a 2.5×10^{-4} M concentration of C14 sulfonate promoted maximum alumina recoveries.

The water/oil volume ratio of 25% contains the minimum oil volume to attain close packing of ideal uniform spheres. Lai and Fuerstenau state that, under these conditions, the possible phase inversion from an oil-in-water to a water-in-oil emulsion is diminished.

As one might anticipate, the more wetting agent added, the more stable and finely divided was the emulsion formed during agitation. When effective concentrations of surfactant were added, the time required for the emulsion to break and separate completely into two layers became a problem. The dispersed oil droplets first coagulated, rose to the top of the water layer to form a cream layer; then the cream layer very slowly coalesced contributing

to the formation of a benzene layer above the cream layer. Complete coalescence of the cream layer sometimes required as long as overnight.

Emulsification and Demulsification – There are conflicting requirements in the TPLS system. Ideally, a finely divided oil in water emulsion must be formed to extract the major quantity of asbestos present, and then this emulsion must break rapidly and completely into a water layer and an oil layer. The more finely divided the emulsion, the more stable it is. The time required for complete separation becomes too long, without some aid to accelerate the process.

Emulsification – As previously described, the first dispersion method employed to form the emulsion was ultrasonic treatment. Then, merely hand shaking was used. In either case, when ≈0.04% of Aerosol OT was added to the water phase or benzene phase, an emulsion was formed that required a standing period of approximately 12 hours to break completely.

Literature[7] indicates that intermittent shaking was more effective in emulsion formation than uninterrupted shaking. Sherman suggests that it requires a small amount of time for the surface-active reagent to be adsorbed on the surfaces of newly formed droplets and to stabilize them fully. Experimentally he found that five intermittent shakings (2 shakes each) with a rest period of 20 to 30 seconds between shakings would completely emulsify 60% by volume of benzene in 1% aqueous sodium oleate. It required 3000 uninterrupted shakes in a machine, lasting about 7 minutes, to completely emulsify the same mixture. First, intermittent shaking was incorporated into the TPLS procedure, and appeared to be reasonably effective. However, Sherman also states that hand shaking results in globules ranging from 50 to 100 μm and that to get smaller droplets one must apply more vigorous agitations to the liquids. It was speculated that more complete extraction of chrysotile would be promoted if the surface contact were increased between the water phase and benzene phase by the creation of smaller oil globules, but ultimately it was found that more complete chrysotile separation was promoted by continual agitation than by intermittent agitation. Continual agitation presumably increases the contact between the water and oil phases by not allowing time for dispersed oil droplets to acquire the stabilizing surfactant layer; the droplets form, coalesce, and reform, and thereby extract more chrysotile from the water phase than when the first droplets formed are allowed time to stabilize.

Demulsification – In the initial stages of development of TPLS, it was obvious that some procedure must be devised to promote rapid and complete demulsification. Consequently, a search was made for a simple, reproducible method of demulsification that would not interfere with the selective extraction of chrysotile into the benzene phase.

In consulting the literature[8], it was found that there are several ways to promote demulsification. Some of the approaches to breaking an emulsion are: addition of excess solvent, addition of a solvent miscible with each of the two phases, destruction of the emulsifying agent, salting out, filtration, heating, freezing, electrolyzing, centrifuging, or changing the pH.

The approaches to demulsification considered most appropriate for application to the TPLS system from the standpoint of simplicity and possible lack of interference were heating, salting out, and changing the pH. Changing the pH was tried first, and it was found that the emulsion

broke at pH 3. In view of the rather high solubility of chrysotile in acid, this approach was abandoned.

Heating the emulsion was tried next, and it was found necessary to heat almost to the boiling point of water to break the emulsion. Heating to such a high temperature caused the benzene phase to boil off explosively evolving large quantities of noxious benzene vapor. However, demulsification by heating has the attraction of not changing the chemical environment; thus successive extractions of chrysotile under similar conditions from the same water sample are possible.

Salting out was the most successful method. G. M. Sutheim[9] states that if a strong electrolyte salt is added, the emulsion coagulates as a result of interference with the adsorbed emulsifying agent at the benzene-water interface. He found the coagulating effect of anions to decrease as follows: citrate, sulfate, acetate, chloride; for cations: sodium, potassium, ammonium, magnesium, calcium, barium. According to this listing, sodium citrate should have the greatest coagulating effect. Therefore, sodium citrate was the first reagent to be tried in a salting out experiment.

Ten ml of 25% sodium citrate was added to an emulsion created by shaking together 50 ml of water containing a suspension of 0.6 mg of chrysotile and 9.2 mg of diatoms and 50 ml of benzene to which 2.0 ml of 1.0% Aerosol OT dissolved in benzene was added. After the addition of sodium citrate the mixture was shaken once and allowed to stand. Within 5 minutes the emulsion broke completely into two layers. The benzene layer was pipetted off and filtered, and a preparation was made for electron microscopy. Both diatoms and chrysotile were found to be present in the benzene fraction. The interference with selective separation was attributed to the organic citrate ion. Consequently, sodium chloride was selected for subsequent trials. Ten ml of 25% NaCl was added to an emulsion formed by intermittently shaking together:

(a) 50 ml of water containing a suspension of 0.2 mg of chrysotile and 5.0 mg of diatoms

(b) 50 ml of benzene to which was added 2.0 ml of 1.0% Aerosol OT in benzene.

After adding the NaCl, the emulsion was shaken again and allowed to stand until it separated completely into benzene and water layers. This required approximately 5 minutes. The benzene layer was then pipetted off and filtered, and a preparation was made for electron microscopy. Only asbestos was found in this preparation indicating that salting out with NaCl did not interfere with TPLS selectivity.

When these developments indicated that TPLS could be speeded up, effort was directed toward TPLS optimization. Attention was focused on the investigation of the identified factors affecting degree of TPLS selectivity and recovery of chrysotile from the water.

The following listed factors were taken into consideration during the experimental studies to establish the best TPLS procedure. Those factors subject to control were varied to search for optimum conditions for TPLS selectivity and recovery.

Factors Affecting TPLS Optimization

(1) Relative interfacial tensions in the solid-liquid-liquid system

(2) Hydrogen ion activity (pH)

(3) Concentration of the surfactant

(4) Oil/water volume ratio

(5) Alkyl chain length on the surfactant

(6) Zeta potential of the particle type

(7) Adsorption density (moles/cm^2) of surfactant surface

(8) Chemistry of the chrysotile surface

(9) Effects of added ions

(10) Agitation conditions

(11) Emulsification.

Experimental Procedures in TPLS Optimization

The criteria used to judge TPLS optimization were degree of recovery and selectivity. Experimental results were evaluated by transmission electron microscopy. In recovery studies, aliquots from a standard chrysotile suspension were subjected to TPLS and recovery was evaluated by comparing the quantity of extracted chrysotile to that in comparable aliquots not subjected to TPLS. By using heavy concentrations of chrysotile (≈4.0 mg/l), it was possible to make rapid comparisons of chrysotile concentrations without resorting to fiber counting. Although this analytical procedure was quite effective, no numbers expressing recoveries were derived. However, when conditions were found that favored maximum recovery, atomic absorption spectrometric analysis (AAS) for magnesium was run to obtain data expressing percentage recovery. Again large quantities of chrysotile were employed to obtain the most sensitive AAS measurement of recovery.

Specimens for TEM recovery analysis were prepared by depositing the chrysotile from an aliquot of the standard suspension on a 0.45 μm, 25 mm, Millipore filter, and depositing the chrysotile separated by TPLS from a similar aliquot on another Millipore filter. A piece was cut from each filter and placed deposit side down on an electron microscope specimen grid bearing a carbon support film. The Millipore filter material was dissolved by the Jaffe Wick Method to prepare a specimen suited to viewing in the TEM where recovery was evaluated by comparing asbestos concentrations at 5000X magnification.

A great many experiments were run before achieving recovery approaching 100 percent. Consequently, the recovery results of most experiments could be quickly judged by electron microscopy. Specimen preparations were saved from the various recovery experiments in order to be able to make comparisons among results as TPLS development progressed.

After TPLS recovery was optimized and combined with light microscopic detection, recovery of those fibers detectable by light microscopy was assessed by comparing light

microscopic counts on as-filtered aliquots to counts made on TPLS fractions from similar aliquots. The results are discussed in the report section dealing with determination of limits of detection.

Selectivity was assessed from the results of TPLS applied to synthetic mixtures of diatomaceous earth and chrysotile as well as from results obtained when TPLS was run on real water samples. Interferences giving both high and low results were assessed. TPLS selectivity was not found to be as great a problem as recovery. Selectivity of the final TPLS procedure for chrysotile in the presence of diatomaceous earth is excellent; only an occasional diatom was found among matted pure chrysotile fibers in the electron microscope preparations.

TPLS selectivity when applied to real water samples is discussed in a later section entitled "TPLS Spot Test Analysis of Real Water Samples". Although interferences giving both high and low results were identified in these studies, they did not prevent chrysotile detection in these samples.

Effects of Critical TPLS Factors on Selectivity and Percent Recovery

The most critical factors in TPLS proved to be (1) mode of agitation, (2) type of surfactant, (3) concentration of surfactant, (4) number of extractions from a single water aliquot, (5) ratio of oil to water phase, (6) pH, and (7) the promotion of the reaction between the chrysotile surface and the surfactant. Although the effects of some factors overshadowed others, all were found to be important in the optimization of recovery and selectivity.

The problems of recovery and selectivity were dealt with separately during experimentation primarily because there was no satisfactory procedure to evaluate recovery when there were large amounts of interfering extraneous particulate in the original water suspension. Consequently, all recovery experiments were run on standard suspensions of pure chrysotile.

The general TPLS procedure in its stage of development at the beginning of these optimization studies consisted of adding anionic surfactant to the water phase, agitating the water phase and oil phase together, salting out the emulsion that formed upon agitation, and after phase separation, filtering the oil phase to concentrate the extracted chrysotile on a filter.

Progress toward optimization of TPLS recovery and selectivity is summarized in the following discussions of effects of the critical factors.

TPLS Recovery

Effects of Agitation –

The mode of agitation is perhaps the most critical of all the investigated factors. When all other factors are optimized, if agitation is inadequate, low recoveries are inevitable.

First success was realized using ultrasonic treatment. Later, hand shaking in a separatory funnel produced good results. After consulting the literature[6], it was decided to attempt to obtain better control of agitation by substituting mechanical stirring for shaking by hand. Lai and Fuerstenau reported ≈95 percent recovery of alumina from water suspension into isooctane

using mechanical stirring and they were able to use it while varying other parameters to work toward optimum recovery. The effects of stirring appeared to be quite reproducible.

After switching to stirring, varying recoveries were obtained; some were quite high but others were low. This variability was attributed to other, possibly unidentified, parameters when the manipulation of identified parameters did not consistently improve results.

Mechanical stirring was performed at speeds of 600, 1200 and 2200 RPM and recovery was found to increase with stirring speed (Table 1). However, stirring speeds of 2200 RPM and above were impractical. We settled on 1600 RPM, ran 18 experiments under identical conditions, and analyzed recovery by atomic absorption analysis for magnesium. A mean recovery of 28% ± 14 was obtained. This degree of recovery was considered to be too low and to have unacceptable reproducibility. Consequently, a superior means of agitation was sought.

At this point, shaking by hand was tried again and better recoveries were found. A mechanical shaker was used and consistently higher recoveries were obtained than by stirring. Then differences were observed as factors other than agitation were varied. Ultimately, a recovery of 74% ± 9.6 was obtained (Table 2) using shaking at 150 cycles per minute for 5 minutes. This was considered adequate for the purposes of the rapid analytical method.

Effect of pH –

Hydrogen ion activity is not extremely critical in the range from pH 3 to 7, and there is no problem in maintaining the pH in this range during TPLS. However, to attain the highest positive charge on the chrysotile surface to promote maximum reaction with the anionic surfactant, the pH of the water phase was adjusted to 3.5 where the zeta potential has the highest positive value. This is accomplished usually by addition of 0.1 N HCl. In all cases, the pH of water samples has been found to be above 3.5.

Effects of Surfactant Compound and Surfactant Concentration –

Only one type of surfactant has been investigated to any extent, this is the anionic alkyl sodium sulfosuccinate. However, two analogous compounds of this type have been investigated. Because of initial success with these compounds, dioctyl sodium sulfosuccinate (Aerosol OT and MO-70) and ditridecyl sodium sulfosuccinate (MT-70), no others were sought.

There was some evidence that MT-70 promoted slightly higher TPLS yields than Aerosol OT, but later experiments indicated comparable recoveries with MT-70 and MO-70, the dioctyl sodium sulfosuccinate supplied by Mona Industries Inc., Paterson, New Jersey 07524. Greater TPLS selectivity was observed while using MO-70. Therefore, MO-70 has been recommended in the finalized TPLS procedure.

Surfactant concentration is critical in TPLS, not only from the standpoint of recovery but also in that too high concentration produces emulsion stability and phase inversion. Therefore, concentration must be carefully adjusted to obtain acceptable TPLS recovery and to avoid emulsification problems.

While employing MT-70, optimized recoveries were observed to occur at concentrations ranging from 1×10^{-3} M to 2.5×10^{-3} M. No phase inversion was detected at 1×10^{-3} M, but it was occasionally seen at the 2.5×10^{-3} M concentration. Recoveries were determined by

TABLE 1. EFFECT OF AGITATION BY STIRRING

Run	Stirring Speed, rpm	Percent Recovery Based on Mg Analysis
31370-93A	600	7
31370-93B	600	7
31370-85	1600	25
32608-1	2200	39

TABLE 2. PERCENT RECOVERY WITH 2.5 X 10^{-3} M MT-70

Sample	% Recovery After Five Consecutive Extractions From Same Sample	pH After Five Consecutive Extractions
1	77	4.5
2	59	4.7
3	81	4.7
4	64	4.3
5	67	4.4
6	54	4.5
7	77	4.5
8	72	4.3
9	86	4.4
10	81	4.5
11	72	4.6
12	69	4.3
13	99	4.4
Mean = 74% Recovery		
Standard Deviation = ±9.6		

AAS analysis for magnesium at these two surfactant concentrations to determine whether there was a significant difference in recovery. Twenty-nine determinations were made at 1×10^{-3} M MT-70 concentration and the recovery was measured to be 63% ± 11. At 2.5×10^{-3} M concentration, it was measured to be 74% ± 9.6 (Tables 2 and 3).

Because no further problems with phase inversion were encountered, the higher surfactant concentration is recommended in the description of the rapid analytical method.

Number of TPLS Extractions From a Single Water Aliquot –

In initial TPLS studies, only one extraction was made from the water aliquot. Upon making several extractions and separately filtering each extracted fraction, it was discovered that the bulk of the chrysotile was not necessarily extracted in the first pass. The TPLS procedure was modified to include three extractions. As further studies were made of TPLS yields, it was again modified to include five passes.

Five extractions from the same water aliquot are recommended in the final TPLS procedure. Under these conditions, 75 percent of the chrysotile is extracted.

Ratio of Oil Phase to Water Phase –

The ratio of the volume of oil phase to the volume of water phase is important with respect to phase inversion. The oil volume was varied from 25 percent of the total liquid volume to 50 percent (1:1 water-oil). We finally settled on 100 ml of water phase and 50 ml of isooctane. Under these conditions satisfactory interfacial contact occurs and phase inversion is, in most cases, avoided.

Promotion of the Reaction Between Chrysotile and Surfactant –

It was found that TPLS recovery was improved by heating the water phase after the addition of the surfactant. Presumably, heating promotes the reaction between the chrysotile surface and surfactant. Perhaps one of the mechanisms by which this occurs is the desorption of interfering species from the chrysotile surface.

Heating at 60°C is recommended in the routine TPLS method.

TPLS Selectivity

Preliminary experiments to investigate TPLS selectivity were run on synthetic mixtures of chrysotile and diatomaceous earth. Diatomaceous earth was chosen because of the prevalence of diatoms in water samples. The recommended method is quite selective for chrysotile in the presence of diatoms.

It is impossible to predict the interferences that might be encountered when applying TPLS to water samples; however, TPLS has been run on water samples from 26 different sources. Some interferences were observed, but they were not extremely serious. All were related to lack of TPLS selectivity. Interferences that could give both high and low results were observed.

High results were obtained on some samples from which organic filaments were extracted by TPLS. Nevertheless, these filaments can be identified as not asbestos with a little experience.

TABLE 3. PERCENT RECOVERY WITH 1.0 X 10^{-3} M MT-70

Sample	% Recovery After Five Consecutive Extractions From Same Sample	pH After Five Consecutive Extractions
1	71	4.8
2	68	4.7
3	61	4.8
4	50	4.2
5	75	4.3
6	75	4.6
7	82	4.7
8	89	4.8
9	82	4.8
10	46	4.4
11	55	4.6
12	82	4.7
13	65	4.8
14	68	4.5
15	71	4.6
16	48	4.6
17	48	4.7
18	63	4.5
19	55	4.6
20	55	4.6
21	55	4.6
22	63	4.7
23	68	4.7
24	51	4.7
25	40	4.8
26	55	4.8
27	55	4.7
28	63	4.8
29	63	4.8

Mean = 63%

Standard Deviation = ±11

These filamentous structures are approximately 0.1 μm in diameter and have varying lengths. One of their identifying characteristics is that along their lengths they have swellings 0.2 μm in diameter and approximately 0.5 μm long. These swellings can be seen in the light microscope with vertical illumination at 500X magnification after the filtered TPLS deposit has been carbon coated. No other interferences giving high results were discovered.

Two types of interferences produce low results. One is an extracted platy mineral that could obscure chrysotile fibers. These plates yield a diffraction pattern the same as that of talc. In no case was a sufficient quantity extracted to prevent the detection of chrysotile.

The other type of interference that will give low results is related to the nature of the chrysotile surface. If magnesium has been leached from the fiber surface, the zeta potential becomes negative, hence, it will not react with the surfactant. Also, coatings may form on the fiber and thus prevent access of the surfactant. Observed in the TEM, these coatings have low electron densities indicating that they are probably organic.

In the cases in which interference from organic material is a problem, heating after surfactant addition improves recoveries of coated fibers, but removal of organic fractions by a chemical treatment possibly would result in further improvement. Because of the time required, low-temperature ashing was not considered during the development of the rapid analytical method. Neither were "wet ashing" techniques explored. It is believed that any future work should include the investigation of "wet ashing".

Handling Large Water Sample Volumes by TPLS

It is desirable to apply TPLS to the particulate in relatively large volumes of water in order to realize the most sensitive detection possible. One of the approaches taken was to increase the volume of water sample handled by TPLS. This resulted in a corresponding increase in the volume of isooctane and an increase in the time required to filter the separated chrysotile. Thus, it appears that scaling up TPLS is not a satisfactory solution.

A scaled-up procedure was used to run 500-ml water aliquots. The filtration time required to filter the 500 ml of isooctane from each of 5 extractions through a 25-mm, 0.2-μm Nuclepore filter using the standard Millipore reservoir was approximately 2-1/2 hours. Two anionic surfactants (MO-70 and MT-70) were used in these experiments. Scaling up to handle the 500-ml aliquots involved the use of a 2000-ml separatory funnel equipped with a Teflon stopcock to avoid stopcock grease. Pure chrysotile was suspended in 500 ml of filtered deionized water; 250 ml of 0.01 M surfactant was added and the suspension was sonicated for 15 minutes. The suspension was heated to 150°F while stirring and subsequently cooled to room temperature. Then, 250 ml of water and 500 ml of isooctane were added. TPLS was performed and the separated fractions were deposited on Nuclepore filters. Those separations using MO-70 (dioctyl sodium sulfosuccinate) were relatively free of NaCl crystals and exhibited good recovery. MT-70 (ditridecyl sodium sulfosuccinate) separations contained a great many NaCl crystals; however, chrysotile recovery was also quite good.

Even if smaller oil-to-water ratios were used, the filtration time would still pose a problem. Filtration and resuspension was found to be a better way of handling large water volumes.

Filtration and Resuspension –

Work has been done to determine the feasibility of filtering relatively large volumes of water, removing the filtered deposit, and resuspending the particulate in smaller water volumes to facilitate the application of two-phase liquid separation. Six different types of filters were used, and removal of the deposits was tried using ultrasonic treatment and shaking.

Standard chrysotile suspensions (200– to 500–ml aliquots) were filtered using MF–Millipore, Duralon, Polyvic, Celotate, Nuclepore, Gelman Metricel (DM 45), and Mitex filters. Fluoropore filters were also tried, but water would not pass through them without the addition of alcohol or other liquid to promote wetting. Ultrasonic treatment disintegrated the MF-Millipore, Duralon, Polyvic, and Celotate filters; however, the best recoveries were obtained by ultrasonic treatment of Nuclepore, Mitex, and Metricel DM 450 filter deposits. Recoveries from Metricel DM 450 and Nuclepore filters were best of all and were judged by microscopic examination to approach 100 percent. Some Teflon fibers were dislodged from Mitex filters by ultrasonic treatment, but, despite this problem, the redeposited chrysotile was quite visible.

Shaking, although not as effective in particle removal, did not break any of the filter types. In all cases, some of the chrysotile was removed, and was found to contain no extraneous debris when redeposited. A means of agitation employing less energy than the particular ultrasonic treatment used and greater energy than shaking would likely make it feasible to remove the major quantity of deposited particulate from any of the filter types.

TPLS did not separate the chrysotile from filter fragments; both were transferred to the isooctane. Chrysotile removed ultrasonically from Mitex, Metricel DM 450, and Nuclepore filters was separated from the water phase by TPLS. Although Nuclepore is one of the two best filters from the standpoint of particle removal, it is fragile and difficult to handle in a field operation. The procedure found most effective for the removal of the filtered deposit in preparation for TPLS was to add 50 ml of 0.005 M MT-70 to the filter, deposit side down, in a 250-ml flask and to sonicate for 15 minutes; 25 ml of deionized water was then used to rinse the filter as it was removed from the beaker, bringing the volume to 75 ml. At this point, the resulting suspension was heated to 150°F while stirring to promote the reaction between the surfactant and the chrysotile surface. Next, the volume was increased to 100 ml by adding deionized water to make the MT–70 concentration 2.5×10^{-3} M; then 50 ml of isooctane was added and the TPLS procedure was carried out. Five extractions were made and the separated fractions were combined on one 0.2-μm-pore-size Nuclepore filter. Evaluations of recovery were made on carbon-coated deposits with the light microscope, or on Jaffe preparations in the electron microscope.

Twenty-four experiments were made in which filter deposits were removed ultrasonically and subjected to TPLS. Good recoveries were obtained.

Metricel DM 450 filters were the best found. They are easily handled in the field, particles are readily removed from the filter surface by ultrasonification, and they do not break up in the ultrasonic bath. The DM 450 filter has a 0.45-μm pore size and it is composed of a PVC copolymer. The diameter of the DM 450 filters used was 47 mm. Larger diameters, of course, could be used to filter larger quantities of water or water containing heavy concentrations of suspended particulate.

If large water samples that are heavily loaded with particulate must be handled, several 47-mm-diameter filters can be used. The collected particulate can be removed ultrasonically in a 100-ml volume of water containing the anionic surfactant.

Filtration and resuspension is recommended as an alternate procedure in the TPLS – light microscopic analytical method. When greater sensitivity is desired than is possible by extracting a 75-ml aliquot, then it is suggested that filtration and resuspension be employed.

Another advantage of filtration and resuspension is that samples can be collected and filtered soon afterwards. This would minimize sample storage problems.

TPLS Recovery as a Function of Particle Size and Aspect Ratio

Experiments were conducted to determine whether TPLS favors the extraction of certain chrysotile fiber sizes and aspect ratios. Several groups of experiments have been run investigating TPLS particle size recovery; however, the set of experiments that used the finalized TPLS conditions are the most pertinent. This last set of experiments also was designed to determine the effects on particle size recovery as a function of zeta potential.

Four samples of chrysotile having different zeta potentials were supplied by Mr. George Reimschussel of Johns Mansville Research and Engineering Center. Mr. Reimschussel measured their electrophoretic mobilities as listed below:

Sample	Electrophoretic Mobility at pH 10
96-1	+3.9 MV
96-2	+0.8 MV
96-3	-1.2 MV
96-4	-2.6 MV

No electrophoretic mobilities were measured at lower pH conditions. Nevertheless, they must all have positive zeta potentials at pH 3.5–4. Good TPLS recoveries were obtained in this pH range. The existence of zeta potential variations among chrysotile types makes it important to control the pH during TPLS.

Standard chrysotile suspensions were prepared from samples 96-1, 96-2, 96-3 and 96-4, and equal (50 ml) aliquots were taken from the same suspension. One aliquot was merely filtered onto a 25-mm, 0.2-μm-pore-size Nuclepore filter; three other aliquots from the same suspension were subjected to TPLS and the extracted chrysotile was also deposited on the same type Nuclepore filters. Specimens for TEM were prepared from the filtered deposits by carbon coating followed by dissolution of the filter material by the Jaffe Wick Method.

The following TPLS procedure was used.

1. To a 75-ml water aliquot adjusted to pH 3.5 with 0.1 N HCl, add 25 ml of 0.01 M MO-70, heat to 140°F while stirring and cool to room temperature.

2. Combine resulting solution with 50 ml of isooctane in a 250-ml separatory funnel.
3. Shake the isooctane and water phase together in the separatory funnel at 150 cycles per minute for 5 minutes.
4. Add 10 ml of 10 percent NaCl and shake gently to break the emulsion.
5. When the phases have separated drain off the water phase completely allowing a small amount of isooctane to drain into the water.
6. Pour the isooctane into a beaker from the separatory funnel avoiding water droplets that may remain in the bottom or on the sides of the separatory funnel.
7. Filter the isooctane onto a 25-mm, 0.2-μm-pore-size Nuclepore filter using the standard filter reservoir.
8. Repeat the extraction procedure four times filtering the isooctane each time through the same filter.
9. Dry filter and coat the deposit side with carbon by vacuum evaporating 1 cm of carbon rod 1 mm in diameter at a distance of 15 cm.
10. Prepare TEM specimens by the Jaffe Wick Method.

Specimens were prepared for electron microscopy by carbon coating the filtered chrysotile deposits and dissolving away the Nuclepore filter by the Jaffe technique. TEM fiber counts and measurements of fiber lengths and widths were made of at least 100 fibers in each preparation. A computer was used to assign lengths, widths, and aspect ratios to size classes and to plot each versus cumulative number percent and cumulative volume percent.

Upon examination of the plots, it was evident that the cumulative volume percent plots were too greatly affected by occasional fibers having unusually large diameters. Also, the widths of fibers extracted by TPLS did not differ significantly from those not subjected to TPLS. Consequently, it was decided to include in this report only two sets of plots for each sample, i.e., length versus cumulative number percent and aspect ratio versus cumulative number percent. Each plot presents the results of four determinations; one of the as-deposited chrysotile, and three of TPLS extractions from comparable aliquots. The plots are designated as Figures 8 through 15.

The results show that TPLS favors the extraction of the longer fibers with larger aspect ratios. In this respect, these latest results are consistent with those previously obtained under different TPLS conditions. This is acceptable in the sense that it is more desirable that the more visible larger fibers are selectively extracted when light microscopic detection is used. In addition, investigators of health effects have reported that the longer fibers are more carcinogenic than the shorter ones.

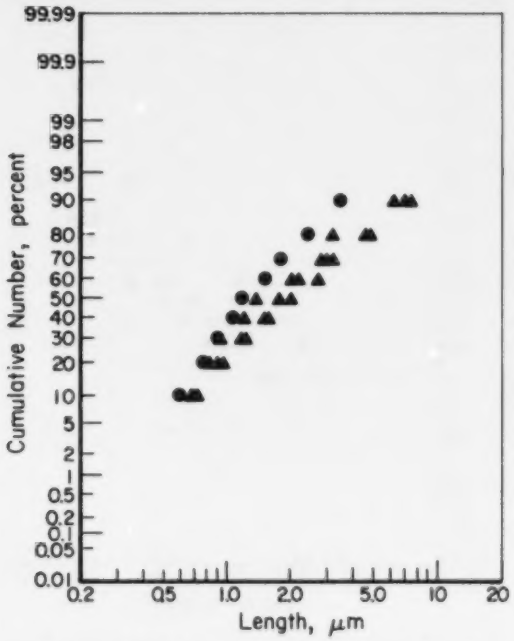


Figure 8. Distribution of chrysotile fiber lengths in sample 96-1 before (●) and after (▲) TPLS.

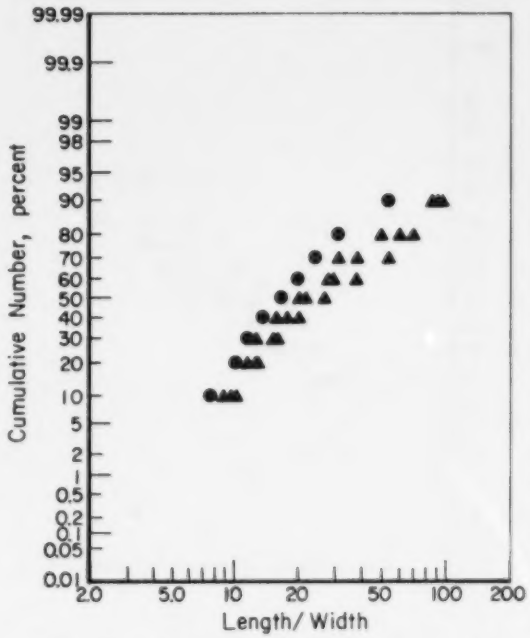


Figure 9. Distribution of chrysotile fiber aspect ratios in sample 96-1 before (●) and after (▲) TPLS.

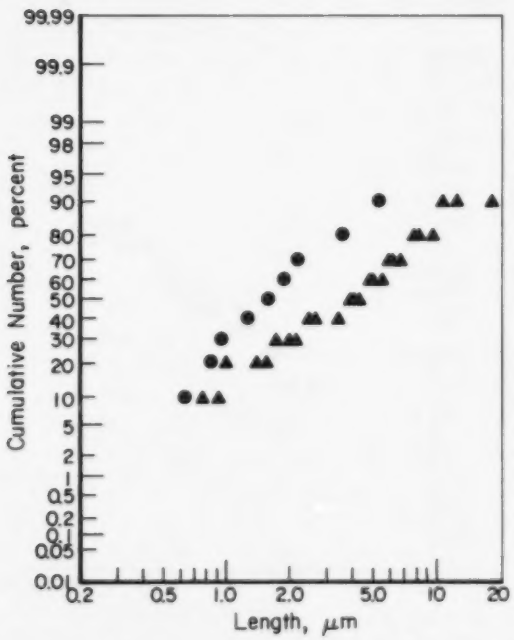


Figure 10. Distribution of chrysotile fiber lengths in sample 96-2 before (●) and after (▲) TPLS.

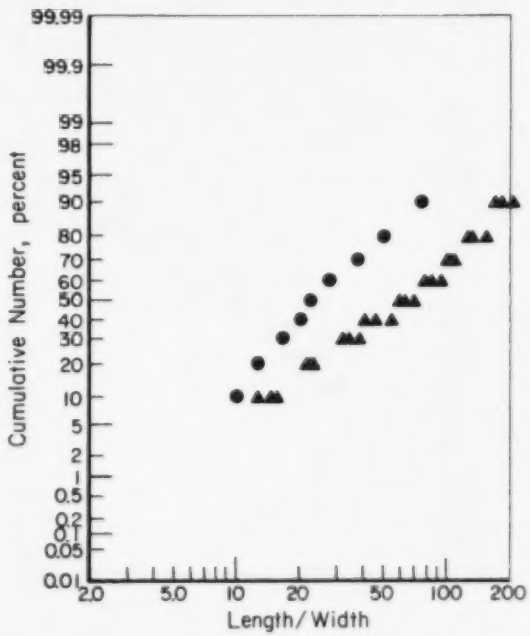


Figure 11. Distribution of chrysotile fiber aspect ratios in sample 96-2 before (●) and after (▲) TPLS.

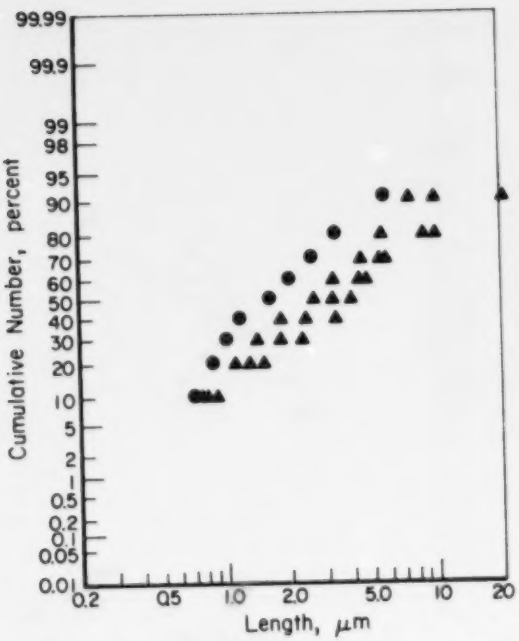


Figure 12. Distribution of chrysotile fiber lengths in sample 96-3 before (●) and after (▲) TPLS.

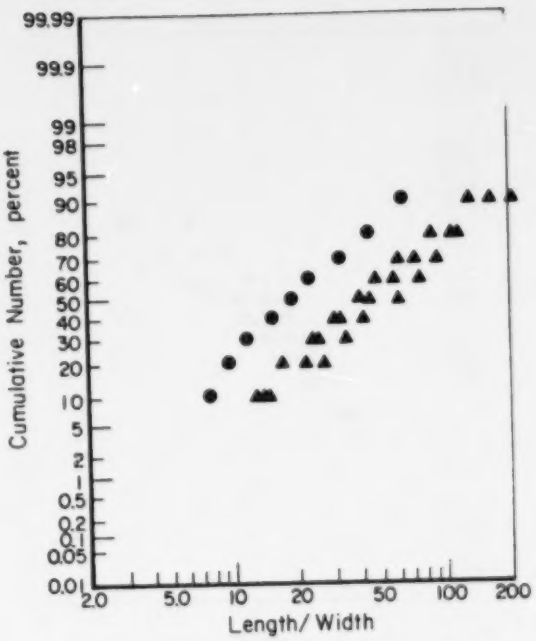


Figure 13. Distribution of chrysotile fiber aspect ratios in sample 96-3 before (●) and after (▲) TPLS.

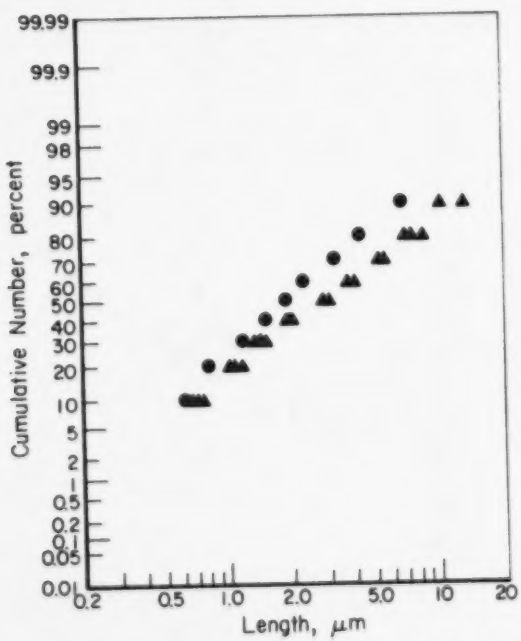


Figure 14. Distribution of chrysotile fiber lengths in sample 96-4 before (●) and after (▲) TPLS.

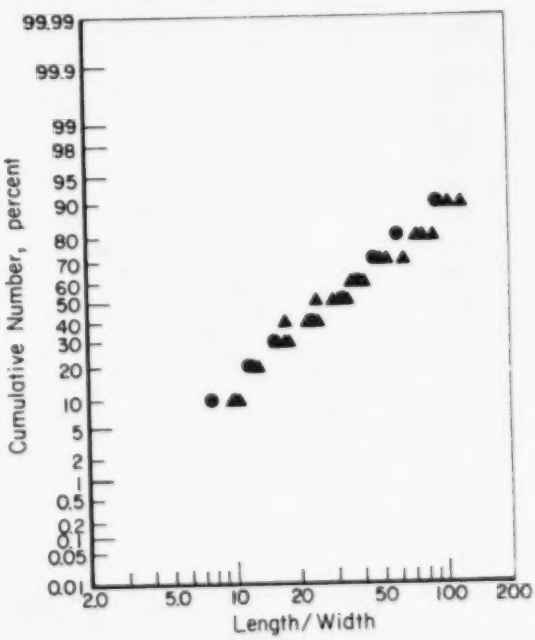


Figure 15. Distribution of chrysotile fiber aspect ratios in sample 96-4 before (●) and after (▲) TPLS.

PHASE II: INVESTIGATION OF DETECTION METHODS

Change of Flow Rate as a Function of Chrysotile Concentration

A survey of detection methods was made in an effort to provide rapid semiquantitative analysis of chrysotile separated and concentrated by TPLS. The methods investigated were change in flow rate of a liquid through a filter as a function of the deposited mass, spot-test procedures, X-ray diffraction, and light microscopy. The objective was to develop a simple routine detection procedure with sufficient sensitivity to detect asbestos concentrations below the significant level in order to eliminate insignificant samples from electron microscopic analysis.

No attempt was made to confine detection to fiber counting. Rather, a search was made for a bulk detector that could be used without the need for a high degree of skill to screen large numbers of water samples rapidly. This was the reason for the investigation of a method so simple as that which attempted to correlate changes in flow rate through a filtered chrysotile deposit with the mass of chrysotile extracted by TPLS.

The flow rate of a liquid through a filtered deposit becomes slower as the mass of deposited particulate becomes greater. It was believed that this phenomenon could provide the basis for a rapid detection method.

Experiments were run to determine the flow rates through measured quantities of chrysotile deposited on 25-mm-diameter Nuclepore (0.1 μm pore size) filters and dried. Initial experiments were run using the standard Millipore 25-mm filtering apparatus.

The measurements were made by introducing 15 ml of isooctane into the reservoir, applying the vacuum, and recording the time required for the isooctane to pass through the filter. The pressure drop was noted for each point.

Quantities of chrysotile ranging from 5.0 to 40.0 μg were used. Both pressure drop across the filter and flow rate were measured. The plot of pressure drop and flow rate versus chrysotile concentration is shown in Figure 16. Pressure drop was not a very sensitive indication of chrysotile concentration. Flow rate was a more sensitive measurement, but not sufficiently so under these experimental conditions.

Further experimentation to obtain more sensitive measurements by flow rate resulted in detection of quantities as low as 0.5 μg. This was accomplished by making a special filter reservoir with a 3-mm hole in order to confine the filtered chrysotile to a smaller filter area, thus increasing the change in flow rate for a given amount of chrysotile.

Attempts to extend detection below 0.5 μg were not successful; furthermore, when analysis of the TPLS fraction from a real water sample was tried, a relatively small amount of interfering platy mineral particles gave too high results. The 0.5 μg detection limit was not considered to be adequate. No further effort was made to use this approach.

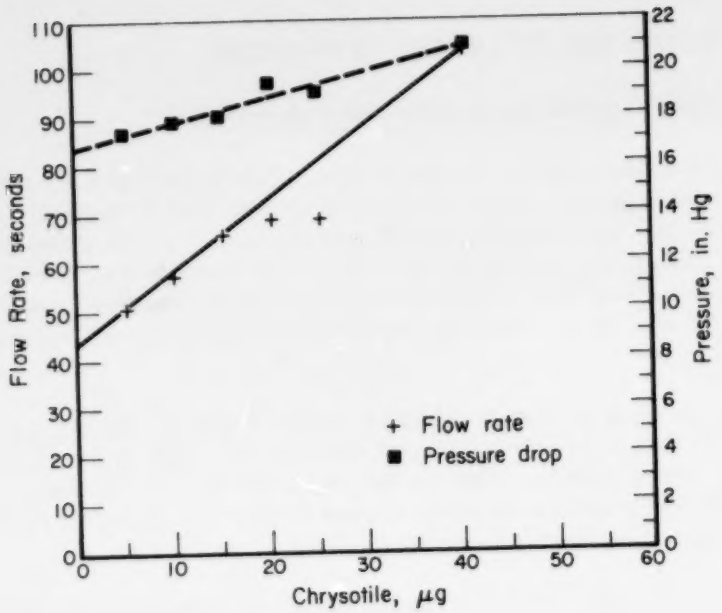


Figure 16. Plot of pressure drop and flow rate vs chrysotile concentration.

Spot-Test Detection

A spot test can be a very sensitive method for detection of small quantities of elements or compounds. F. Feigl in his book entitled *Spot Tests, Inorganic Applications* has described chemical reactions that produce colored reaction products. Both qualitative and quantitative results can be obtained. When a selective reaction occurs, characteristic color is generated and often the depth of the color can be correlated with concentration.

Chrysotile containing only magnesium, silicon, and oxygen, as do many other minerals found in water, cannot be selectively detected on the basis of the presence of these elements. However, it does have some relatively unique reactivities. It will adsorb iodine vapors and it also will feature in ion exchange reactions. The magnesium can be replaced by other cations as environmental conditions are changed to promote substitution. This property was exploited in the development of a spot test procedure for chrysotile detection.

Manganous ion will react with chrysotile and can be detected very sensitively by means of a spot test reaction that is catalyzed by manganese. According to Feigl, as little as one nanogram of manganese can be detected by the reaction between potassium periodate and tetrabase (tetramethyl-4–diaminodiphenylmethane). Tetrabase is the leuco form of a diphenylmethane dye that, in the presence of manganese, is oxidized to a bright blue compound by periodate.

The detection procedure involves (1) filtration of chrysotile separated by TPLS using a filter reservoir with an ≈3-mm-diameter hole to confine the deposit to an ≈4-mm-diameter area, (2) reaction of the chrysotile with manganous sulfate, (3) washing to remove unreacted $MnSO_4$, and (4) detection of reacted manganese by the periodate-tetrabase spot test.

The detailed spot-test procedure is as follows:

1. Chrysotile as extracted by TPLS is deposited on a 25-mm, 0.45-μm-pore-size Millipore filter in an ≈3-mm-diameter area and dried. This is done by using a special reservoir with a 3-mm-diameter opening.
2. The special reservoir is then removed and the filter bearing the deposited chrysotile is covered with a Millipore filter, and the standard 25-mm reservoir is used for Step 3.
3. Fifteen ml of 25% $MnSO_4 \cdot H_2O$ at room temperature is poured into the filter reservoir and aspirated through the deposit over a period of 1 minute.
4. Next, the filtered deposit is washed with 15-ml quantities of room temperature deionized water 5 times aspirating each washing completely through the filter.
5. The filter reservoir is removed while applying suction to the filter to hold it to the frit and then the covering Millipore filter is removed with tweezers.
6. The filter bearing the chrysotile deposit is removed and dried under a heat lamp at 145°F (63°C).
7. The filter is attached by the edge to a piece of white blotter paper deposit side up and sprayed with the spot testing working solution and placed under the heat lamp at 145°F until development is maximized (≈2 minutes).
8. The depth of blue color that develops is compared to photographs of a series of standards made using known quantities of chrysotile.

The working spot test solution is made up by combining equal volumes of the Solutions A and B:

Solution A: 1.0% solution of tetrabase in 95% ethyl alcohol.

Solution B: A saturated solution of potassium periodate in 2N acetic acid.

Comparison of the depth of color of the spot test result is possible over a chrysotile concentration range from 0.1 μg to ≈0.8 μg. At 0.8 μg and above the color is so intense that little change with increase in concentration can be detected. Nevertheless, when used primarily to select samples for TEM analysis the lack of good quantitative indications above 0.8 μg poses no problem.

The spot test procedure has the advantage that it can be carried out quickly with a minimum of expensive apparatus and training; but the preparation, unlike that used for light microscopic detection, cannot be readily used for electron microscopy when confirmation of results is desired.

X-Ray Diffraction

The techniques of powder X-ray diffraction were explored in an effort to establish a detection procedure. The primary difficulties were quantification and sensitivity of detection.

The X-ray powder camera was used in most of the experiments; diffractometer methods were not considered to have sufficient sensitivity. The major problem was with specimen

preparation when dealing with lower quantities of chrysotile (<30 ng). No way was found to prepare a specimen so that it would be entirely irradiated by the X-ray beam.

Light microscopy was found to be far superior to X-ray diffraction. Fibers detectable by light microscopy have always been found in water samples containing chrysotile even when the mean fiber length is below 1.0 μm.

Light Microscopy

It was found that light microscopy employing vertical illumination as obtained with metallographic optics is an extremely sensitive means of detecting fiber shapes in a carbon-coated TPLS deposit on a Nuclepore filter. When interferences from particulate other than chrysotile are virtually eliminated by TPLS, the separated chrysotile fibers are quite visible. The carbon coating increases the detectability of the chrysotile by two mechanisms; it enhances image contrast by increasing the reflectivity of the Nuclepore substrate and also increases the apparent fiber diameter to the point that some of the fibers with diameters too small to be seen by light microscopy become detectable if their lengths are greater than ≈1.0 μm. The fibers are seen as dark structures against a bright background because the microscope illumination is along the axis of the objective lens. The Nuclepore filter surface is perpendicular to the objective axis reflecting light directly back into the objective and the fiber surfaces are not perpendicular reflecting light away from the objective.

A quick examination with the light microscope is all that is required to determine whether a filtered chrysotile deposit is greater than 100 nanograms. Analysis of intermediate concentrations is performed with an eyepiece reticule having 10 concentric circles to minimize fiber counting time as fiber concentration varies (Figure 17). When concentrations are relatively high, counts are made within circles near the center, and lower concentration counts are made within circles of greater diameter – sometimes across the diameter of the filter deposit.

Light microscopic detection of chrysotile was placed on a quantitative basis by generating a standard curve relating chrysotile mass per filter to counts made at 500X magnification while viewing a carbon-coated Nuclepore filter with vertical illumination. Counts are made while scanning a 250-μm-wide path across the diameter of the effective filter area. The eyepiece reticule having 10 concentric circles spaced 12.5 μm apart is employed (Figure 17), and one count is registered for a fiber segment equal to the spacing between concentric circles. Compensation for variations in fiber width are made by estimating the number of minimum detectable fiber widths in larger fibers or bundles. Because the outermost circle delineates a 250-μm-diameter field at 500X magnification, it is used to determine the path width over which counts were made. Counts are made only on fibers falling within that field as the path is traversed.

Standard chrysotile suspensions were filtered onto Nuclepore filters using the standard 25-mm Millipore filtering apparatus. Concentrations ranging from 1.0 nanogram to 10,000 nanograms were deposited on Nuclepore filters, which were subsequently taped flat on microscope slides and coated with carbon. At least six filtered deposits were prepared at each of seven concentrations from two sources of pure chrysotile.

Two persons have made counts on several of these preparations and the reproducibility between the two sets of results appears to be quite acceptable as demonstrated by the agreement

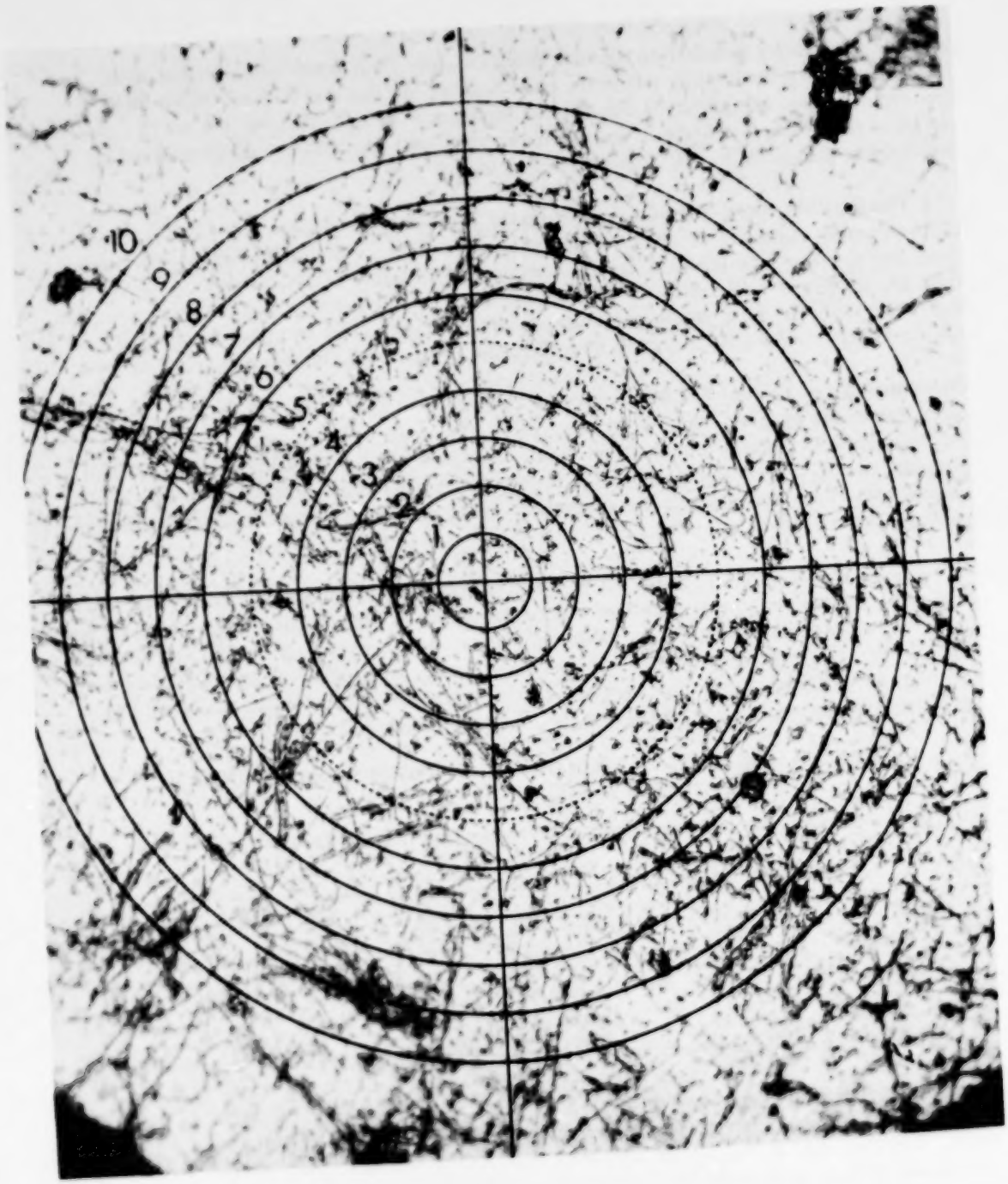


Figure 17. The eyepiece reticule used in fiber counting.

of data. Counting a preparation requires approximately 15 minutes when dealing with 100-nanogram concentrations where the total number of counts is ≈1500. When higher concentrations are encountered, various sized fields may be selected from the set of concentric circles in the eyepiece reticule to count smaller areas and the data may be normalized by multiplying the results by the appropriate area factor.

The standard curve that was thus generated is presented in Figure 18. Counts versus chrysotile mass per filter are shown on a log-log plot. The sensitivity of light microscopic detection appears quite adequate for the purposes of the rapid analytical method. Also, the time per analysis is sufficiently short, especially if the method is to indicate only if a sample should be analyzed more precisely by electron microscopy. Then an upper count limit may be set according to allowable chrysotile concentration to minimize the required counting time.

Determination of Percent TPLS Recovery of Particle Sizes Detectable by Light Microscopy –

The same preparations used to determine the recovery of chrysotile by TPLS by TEM methods were also used to determine the recovery of particle sizes detectable by light microscopy. The counting procedure described in the preceding section was applied to comparable aliquots before and after TPLS.

Results are presented in Table 4. The percent recovery as determined from three separate runs from each of the four samples are quite comparable to the percent recovery as determined by atomic absorption spectrometry. When all results were combined, the mean and standard deviation were calculated to be 75% ± 15. Consequently, when applying light microscopic detection to TPLS fractions, it is suggested that a factor of 2 be applied to the counts made with the light microscope to compensate for fiber loss during TPLS.

TABLE 4. PERCENT TPLS RECOVERY OF PARTICLES DETECTABLE BY LIGHT MICROSCOPY

Sample	Run 1	Run 2	Run 3	Mean ± Std. Dev.
96-1	92	83	65	80 ± 13
96-2	76	92	72	80 ± 10
96-3	97	57	49	67 ± 25
96-4	74	64	81	73 ± 9

Limits of Light Microscopic Detection –

Limits of detection have been determined at 80, 90, 95, 98 and 99 percent confidence levels. The data used in the calculations were obtained by the light microscopic counting technique. Counts were made on 22 different concentrations ranging from 0.8 to 1000 nanograms. An average of approximately six preparations at each concentration were counted. These data were analyzed by a computer program to determine the limits of detection of chrysotile per Nuclepore filter deposit. Table 5 presents the detection limits at 100% TPLS recovery and also at 75% ± 15 TPLS recovery. The latter recovery was determined by a comparison of light microscope counts made on as-deposited aliquots versus similar aliquots from

the same standard suspensions subjected to TPLS. At 60% recovery (75% – 15) the limit of detection at the 99% confidence level was computed to be 1 nanogram per filter deposit.

TABLE 5. DETECTION LIMIT DETERMINATIONS

Percent Confidence Level	Detection Limit (μg) at 100% Recovery	Detection Limit (μg) at 75% ± 15 TPLS Recovery		
		75%	+15	-15
80	2.2×10^{-4}	2.9×10^{-4}	2.4×10^{-4}	3.7×10^{-4}
90	2.7×10^{-4}	3.6×10^{-4}	3.0×10^{-4}	4.5×10^{-4}
95	3.4×10^{-4}	4.5×10^{-4}	3.8×10^{-4}	7.5×10^{-4}
98	5.3×10^{-4}	5.7×10^{-4}	4.8×10^{-4}	2.6×10^{-4}
99	5.2×10^{-4}	6.9×10^{-4}	5.8×10^{-4}	8.7×10^{-4}

The data points used in the calculation of detection limits are tabulated in Table 6. These data were also used to generate the standard curve.

PHASE III: APPLICATION OF ANALYTICAL PROCEDURES

TPLS – Light Microscopic Analysis of Real Water Samples

Seventeen water samples were obtained from Dr. Ian Stewart of W. C. McCrone Associates and from Dr. Eric Chatfield of Ontario Research Foundation. All were subjected to TPLS and the extracted fractions were analyzed by the light microscopic counting technique (see Figure 18), and some of them were analyzed by the spot test procedure. Specimens from these samples were also examined by TEM before and after TPLS to determine whether chrysotile was present and the nature of particulate extracted by TPLS along with chrysotile.

In the as-received condition, many of the samples contained large quantities of extraneous particulate from which it was necessary to separate the chrysotile. First, TEM comparisons were made between two-phase liquid separations from 50-ml aliquots and filtered deposits prepared from 10-ml aliquots of the as-received samples to determine whether there was agreement between the presence and concentration of chrysotile in the as-received sample and the corresponding TPLS fraction. The same preparations were also examined by light microscopy to evaluate the feasibility of using the light microscope for rapid screening in a detection procedure.

Most of the samples had a pH of 7 or higher, which is too high to obtain optimum TPLS results. Consequently, the pH of the 75-ml aliquot was adjusted to pH 3.5 with 0.1 N HCl where the positive zeta potential of chrysotile is maximized. Then, 25 ml of 0.01 M MO-70 was added, the sample heated to 140°C, cooled to room temperature, and 50 ml of isooctane added. TPLS was then carried out and the extracted chrysotile was deposited over a 16-mm-diameter filter area.

TABLE 6. LIGHT MICROSCOPIC DETECTION OF CHRYSOTILE AT TWENTY CONCENTRATIONS

Concentration (μg)	Avg	Counts											
0.00079	5.67	6	6	5									
0.001	8.8	10	6	7	8	5	8	13	11	9	11		
0.0018	31.7	40	29	6	29	38	48						
0.005	81.8	78	72	93	88	86	86	84	70	69	102	79	75
0.0079	93.3	62	91	118									
0.009	157.3	137	128	186	164	137	192						
0.01	119.2	139	139	95	77	116	149						
0.018	260.3	228	272	386	210	238	228						
0.02	272.4	338	294	286	251	225	254	259					
0.025	235.5	225	244	264	231	259	190						
0.036	350.5	410	368	310	296	392	327						
0.045	527.8	583	486	522	500	590	486						
0.079	959.7	972	1,076	831									
0.1	1356.3	1,435	1,064	1,433	1,316	1,600	1,290						
0.18	1679.8	1,708	1,924	1,852	1,780	1,146	1,669						
0.2	2188	1,951	2,425										
0.36	6908.3	7,298	8,528	5,166	4,592	6,658	7,298	5,986	7,462	6,806	8,118	8,364	6,624
0.4	5953.2	6,232	5,904	5,904	5,986	5,740							
0.72	12910.4	13,202	12,218	11,726	13,858	11,070	15,744	12,628	12,464	13,284			
0.79	12426.7	16,705	10,707	9,868									
0.8		10,496	7,954	13,366	11,726	12,628	10,414	9,184	13,694	9,184	13,038		11,168.4
1.0		12,300	13,776	12,710	16,400	11,480	12,054	13,448	16,646	15,990	17,056	15,006	14,260.5

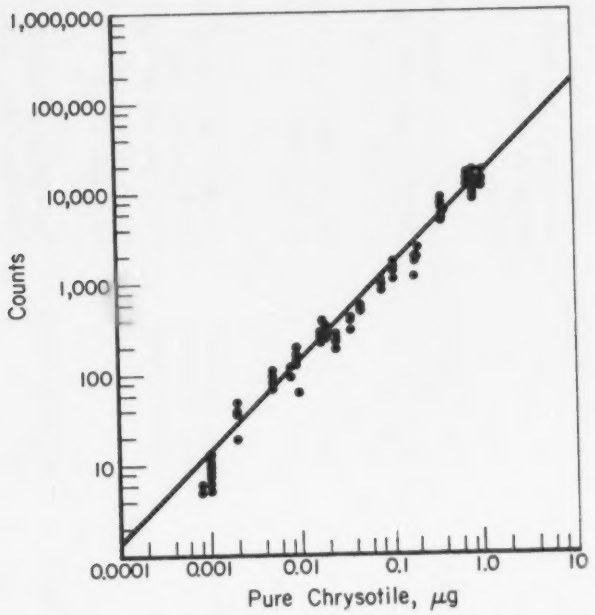


Figure 18. Standard curve that relates light microscope counts to mass (μg) of chrysotile per filter.

Results are presented in Table 7. In general, the results obtained were as follows.

1. In no case was chrysotile found in the as-received preparation and not found in the TPLS fraction by TEM and light microscopy.
2. In five cases, chrysotile was found in the TPLS fraction and not in the as-received preparation; this was attributed to masking by extraneous particulate.
3. Chrysotile was detected in only two of the as-received preparations by light microscopy. This was also the result of masking by other particulate.
4. Filamentous structures, which appear to be organic when seen in the TEM, are present in four of the TPLS fractions. To the inexperienced observer using light microscopy, these filaments might be identified as chrysotile to give false positive results.
5. In two cases, some interference was encountered from other particulate being transferred along with chrysotile to the isooctane; however, chrysotile was detected more readily by TEM and LM in TPLS preparations than in the filter deposits of the corresponding as-received samples.
6. Detection of chrysotile in TPLS fractions from real water samples was found feasible using the light microscope.
7. Light scattering, as seen with the unaided eye, from carbon-coated deposits of the TPLS fractions were observed to correlate fairly well with the presence of chrysotile. False positive results are more likely than false negative results when relying on light scattering.

TABLE 7. RESULTS OF TPLS APPLIED TO REAL WATER SAMPLES

Water Sample Number	Observation in TEM of TPLS Fraction	TEM Observations of As-Received Sample		LM Observations of TPLS Fraction	Remarks
		Debris	Chrysotile		
C3 0514 1530 U3 WGO 11/17 I (filter sample WG3)	Long filamentous organic structures present; very little chrysotile seen	Heavy	Little	Long filaments seen, but no chrysotile	With some experience, these filaments would not be construed as chrysotile when seen with the light microscope
C3 0514 1530 D3 WGO 11/17 II (filter sample WG3)	Many bacteria-like structures plus long organic filaments. Medium amount of chrysotile	Medium	None	Medium number of fiber-shared structures seen	Both chrysotile fibers and organic filaments are visible in the light microscope
C9 0305 1215 U4 WGO 11/17 III (filter sample WG3)	No chrysotile, a few organic filaments, and very little extraneous particulate	Little	None	Very few fiber shapes	Not a significant population of fiber shapes
C9 0305 1350 U5 WGO 11/22 I (filter sample WG 1)	Many clumps of chrysotile, a fairly clean separation containing a few bacteria	Medium	None	Many individual fibers and several clumps of fibers detected	Relatively high fiber population detected by light microscopy indicating possibly significant quantity of chrysotile
C9 0305 1340 D5 WGO 11/17 IV (filter sample WG3)	A clean separation containing many short chrysotile fibers	Little	None	Many short fibers seen	The medium to high concentration of short fibers was unique to this particular sample; despite this characteristic, many fibers were detected by light microscopy
P4 0212 0920 D5 WGO 11/15 II (filter sample WG2)	Very few chrysotile fibers; some extraneous organic particulate	Very heavy	None	Very few fibers seen	No significant number of fibers shapes were seen by light microscopy

TABLE 7. (Continued)

Water Sample Number	Observation in TEM of TPLS Fraction	TEM Observations of As-Received Sample		LM Observations of TPLS Fraction	Remarks
		Debris	Chrysotile		
M3 0307 0730 D1 WIO 11/15 I (filter sample WIe)	A very clean heavy mat of chrysotile fibers too thick to penetrate with electron beam except at edge of filtered deposit	Heavy	Heavy	Mat of fibers	The TPLS fraction when deposited on the Nuclepore filter contained so many fibers that individual fibers could be discerned only at the edge of the deposit
M 3 0307 1500 D2 WGO 11/15 IV (filter sample WG2)	A heavy mat of clean chrysotile fibers too thick to penetrate with electron beam except at edge of deposit	Medium to heavy	Very heavy	Heavy mat of fibers seen	A very dense chrysotile mat from the TPLS fraction in which individual fibers were evident only at the edge of the filtered deposit
P1 0130 0555 D2 WIO 11/15 III (filter sample WI2)	A little extraneous organic particulate; very few chrysotile fibers	Medium	None	Almost no fibers seen	No significant numbers of fiber shapes seen in the light microscope
R2 0305 0615 D1 WIO 11/10 III (filter sample WI3)	A clean separation containing a few long chrysotile fibers; very little extraneous particulate	Very heavy	Very few (other fiber types present)	A few long fibers	Although the as-received sample contained other fiber types than chrysotile, only chrysotile was detected in the TPLS fraction
S1 0326 0700 D4 WIO 11/10 IV	A heavy mat of chrysotile coated with a structureless organic film	Very heavy	None visible	Heavy mat of fibers plus heavy extraneous particles	This sample is one of two in the sample set that contained a large quantity of interfering material in the TPLS fraction

TABLE 7. (Continued)

Water Sample Number	Observation in TEM of TPLS Fraction	TEM Observations of As-Received Sample		LM Observations of TPLS Fraction	Remarks
		Debris	Chrysotile		
GI 0505 0450 D4 WIO 11/8 II 11/22 II (filter sample WI3)	No chrysotile and very few extraneous particles	Heavy	None	A few fiber shapes seen	The few fiber shapes detected in the light microscope gave a weak false positive result
N2 0302 1805 D3 WGO 11/10 II (filter sample WG1)	No chrysotile found, but organic filaments were present	Medium	None	A few fiber shapes seen	A weak false positive result
N8 0403 1730 D6 WGO 11/10 I (filter sample WG2)	No chrysotile found, but organic filaments were present	Heavy	None	A few fiber shapes seen, but not construed as chrysotile	Although fiber shapes were detected in the light microscope, they did not have the appearance of chrysotile
N1 0606 1335 D2 WGO 11/29 I 11/8 I (filter sample WG2)	A medium to heavy amount of chrysotile, also some platy mineral particles	Heavy	None	Many fibers detected	A case in which the debris was so heavy in the as-received preparation that no chrysotile was detected despite a relative high chrysotile concentration
Chatfield No. 1 (US sample)	Gross numbers of chrysotile fibers and bundles of fibers; a clean separation	Heavy	Medium	Dense population of chrysotile	A very good separation of gross amounts of chrysotile
Chatfield No. 2 & 3 (Canadian sample)	Many chrysotile fibers plus several diatoms and other unidentified structures transferred along with chrysotile	Very heavy	Heavy	Heavy fiber concentration plus diatoms and other particles	This is the other sample of two in the sample set that contained a fairly heavy concentration of interfering particulate in the TPLS fraction

8. TPLS procedures will extract wide ranges of chrysotile concentrations from real water samples. Some extractions resulted in mats of fibers too thick to see through with either the TEM or LM, whereas, others contained only a few or no fibers.

It was concluded from this study of the application of TPLS to actual water samples, that TPLS is an effective approach to more sensitive detection of chrysotile by TEM or LM in water samples than in transmission electron microscopy applied to unashed filtered deposits of as-received samples. This is true even under the conditions of incomplete TPLS chrysotile recovery. Also, light microscopy detected extracted chrysotile from samples in which both LM and TEM examination of as-received filtered deposits revealed none.

Light microscopic counts were made on the carbon-coated TPLS deposits on the 0.2-μm-pore-size Nuclepore filters. Although possible interferences existed in a few samples in the form of a platy mineral and filamentous organic structures that might be identified as chrysotile by an inexperienced observer, they did not obscure the presence of significant quantities of chrysotile or contribute to erroneously high counts. The results are presented in Table 8.

Four samples contained gross amounts of chrysotile making it impossible to perform counting. An example of the concentration of fibers is shown in Figure 19. This is a photomicrograph taken at the edge of the filtered deposit extracted by TPLS. TPLS can handle these large quantities without difficulty.

TPLS-Spot Test Analysis of Real Water Samples

The periodate-tetrabase catalytic spot test procedure, as previously described, was applied to TPLS fractions filtered onto Millipore filters. The deposit was confined to a 4-mm-diameter area by using the filter reservoir with a 3-mm-diameter hole. All deposits were dried before reacting the chrysotile with manganous ion.

Figure 20 shows a comparison between spot test results on standard chrysotile concentrations and on chrysotile concentrations extracts from 50-ml sample aliquots by TPLS of spot test analyses of four real water samples.

In cases where no chrysotile was indicated by the spot test, some extraneous particulate was observed. The color prints shown are not the same hue, and it is difficult to obtain prints which are comparable. The positive transparencies from which the prints were made, however, have the same hue and show differences in depth of color better. In view of this, it is recommended that color transparencies of standards be viewed on a light box when comparisons of analytical results from real water samples are made to standards in the filter deposit indicating that the spot test procedure was selective for chrysotile in these cases.

Table 9 presents results of the estimated chrysotile concentrations in nine water samples.

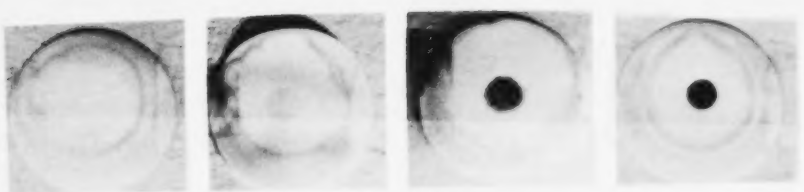
TABLE 8. LM ANALYTICAL RESULTS ON REAL WATER SAMPLES

Sample	Light Microscope Counts	Nanograms per Filter	Nanograms per Liter
C9 305 1215 U4 WGO	0	0	0
N2 0302 1805 D3 WGO	0	0	0
N1 0606 1335 D2 WGO	439	32	640
M3 0307 1500 D2 WGO	Too many fibers to count	>500	>10,000
S1 0326 0700 D4 WIO	Too many fibers to count	>500	>10,000
M3 037 0730 D1 WIO	Too many fibers to count	>500	⩾10,000
P1 0130 0555 D2 WIO	134	10	200
P4 0212 0920 D5 WGO	78	6	120
R2 0305 0615 D1 WIO	85	6.8	136
C9 0305 1340 D5 WGO	Too many to count	>500	>10,000
C9 0305 1350 U5 WGO	705	50	1,000
G1 0505 0450 D4 WIO	0	0	0
N8 0403 1730 D6 WGO	0	0	0
C3 0514 1530 D3 WGO	382	21	420
C3 0514 1530 U3 WGO	14	1.2	24
Chatfield Sample 3 (American Source)	7,926*	400	5,300
Chatfield Sample 1 (Canadian Source)	594,500*	28,000	373,000

*For Sample 3, three fields within #10 ring in the concentric circle eyepiece reticule were counted; for Sample #1, one field delineated by ring #2 was counted.

Figure 19. Light micrograph showing a heavy mat of TPLS-extracted pure chrysotile from sample M3 0307 1500 D2 WGO.

Series of standard chrysotile concentrations



Spot test results on real water samples

Figure 20. Comparison of spot test results on real water samples to spot test results on a series of chrysotile standards.

TABLE 9. SPOT TEST RESULTS ON REAL WATER SAMPLES

Sample	Estimated Concentration (μg) Per Filter
P1 0130 0555 D2	<0.1
M3 0307 1500 D2	>1.5
R2 0305 0615 D1	<0.1
C9 0305 1215 U4	<0.1
M3 0307 0730 D1	>1.5
C9 0305 1350 U5	≈0.1
C9 0305 1340 D5	≈0.5
C9 0305 1215 U4	<0.1

SECTION 5

INTERLABORATORY ANALYSIS OF FOUR WATER SAMPLES

Four water samples supplied by Dr. Eric Chatfield of the Ontario Research Foundation (ORF) were analyzed at both Battelle and ORF by the TPLS-light microscopic analytical procedure.

Instructions describing the procedure were supplied ORF by Battelle. The first attempt at ORF to apply the technique as described revealed that the instructions required more clarification. In particular, the isooctane remained cloudy after the separation procedure, which led the ORF group to believe that they were not following the correct procedure. Cloudy isooctane is normal, however. Another problem concerned the precise conditions for the optical microscope count. As initially planned, Dr. E. J. Chatfield and Ms. M. J. Dillon visited Battelle's Columbus Laboratories to obtain firsthand experience with the technique before attempting analysis of the interlaboratory samples.

The samples supplied by ORF were from Thetford Mines, Quebec; Toronto, Ontario; Asbestos, Quebec; and Lloyd Lake, Ontario. Both heavily loaded and lightly loaded samples, which also contained some extraneous debris, were provided. By electron microscopy, ORF estimated these samples to range from about 100 μg/liter to 10 ng/liter of chrysotile asbestos. Battelle did not analyze these samples by electron microscopy.

At ORF, some deviation from conditions in the instructions was necessary because of equipment and supply limitations. In particular, Nuclepore filters were of 0.1-μm pore size, and backing Millipore filters were of 0.45-μm pore size. All shaking was by hand, and double-distilled water was used instead of deionized water.

ORF detected some artifacts on the surface of some blank Nuclepore filters that might be mistaken for chrysotile fibers. They state that in many cases the artifact can be recognized for what it is: a glancing perforation. In others there is some difficulty in rejecting it as a genuine fiber. No comparable artifacts were observed at Battelle.

The ORF researchers concluded that the two-phase liquid separation technique is a "most effective chrysotile extraction method". They report that the technique allowed processing of much larger volumes of dirty water samples than was possible by direct TEM methods. Also, as Battelle reports, extraction into the organic phase is not totally specific to chrysotile, which sometimes gives rise to difficulties in interpretation of the optical fiber counting. Dr. Chatfield and Ms. Dillon conclude, however, that an experienced microscopist could in many cases distinguish the chrysotile from the other debris.

Specific suggestions were made by ORF to assist in clarification of TPLS-light microscopic analytical instructions. These have been heeded and the instructions modified accordingly.

A general suggestion was made concerning the requirements for optical microscopy. ORF was unable to duplicate the exact light microscope magnification conditions employed at Battelle. The distance between adjacent concentric circles of the eyepiece reticule was 10.0 μm with their microscope rather than 12.5 μm as with the Battelle microscope. Consequently, Dr. Chatfield suggested that the standard curve relate total fiber length to chrysotile mass instead of numbers of 12.5-μm lengths to chrysotile mass. Total fiber length is independent of the features of a particular microscope, so long as an adequate magnification is in use. The eyepiece reticule in any microscope then need only be calibrated and measurements expressed as total fiber length.

Table 10 presents the comparison of the ORF and Battelle results of analyses on four water samples. Agreement is approximately within a factor of 4 in all cases except for Sample 4, in which the Battelle result is ≈0.1 that of ORF. In this case, the Battelle result agrees with the electron microscopy result obtained by ORF. According to results from both laboratories, all of these samples would be destined for more accurate analysis by electron microscopy, with the possible exception of Sample 4.

The results of this interlaboratory comparison emphasizes the need for more extensive round robin testing of the methodology to lead to further improvement of the reliability of results.

TABLE 10. COMPARISON OF ANALYTICAL RESULTS OBTAINED AT BATTELLE AND ORF ON FOUR WATER SAMPLES

Sample Number	Sample Details: Source	Water Type	Description	Estimated Mass by TEM (μg/liter)	Optical Counts: Number of 10.0 μm Segments	Optical Counts: Area Examined (mm^2)	Equivalent Number of 12.5 μm Segments	Number of 12.5 μm Segments Equivalent to Diameter Scan	Mass of Chrysotile (μg/filter)	Aliquot Volume (ml)	Calculated Mass of Chrysotile (μg/liter)
1	Lloyd Lake Ontario	Raw	Sand, Clay	50	233	0.0785	186	9478 ORF 4113 Battelle	0.60 0.28	50 75	16 3.7
3	Thetford Mines Quebec	Treated	Municipal water General debris	1.0	830	3.20	664	830 ORF 5480 Battelle	0.065 0.35	75 75	1.16 4.7
4	Toronto Ontario	Raw	Lake Ontario	1.3×10^{-2}	92	3.20	74	92 ORF 9 Battelle	0.0085 0.0013	75 75	0.15 0.0177
5	Asbestos Quebec	Treated	Municipal water Fine sand	8.0×10^{-2}	234	3.20	187	234 ORF 668 Battelle	0.020 0.050	50 75	0.53 0.66

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APPENDIX A

INSTRUCTIONS FOR CARRYING OUT TWO-PHASE LIQUID SEPARATION COUPLED WITH LIGHT MICROSCOPIC DETECTION OF CHRYSOTILE IN WATER SAMPLES

The rapid analytical method developed at Battelle-Columbus for determining chrysotile asbestos in water involves first the separation of chrysotile from other waterborne particulate followed by quantitative analysis of the separated fraction by light microscopy. Separation is accomplished by a procedure in which the selective transfer of chrysotile from water to isooctane is promoted by a reaction with an anionic surfactant. Subsequently, the separated chrysotile is deposited on a Nuclepore filter and its concentration is determined by a light microscopic counting technique.

The separation procedure is called two-phase liquid separation (TPLS). The selective transfer of chrysotile occurs when the isooctane and water are shaken together in a separatory funnel after the chrysotile surface is rendered hydrophobic through a surface reaction with an anionic surfactant MO-70, dioctyl sodium sulfosuccinate. The presence of the surfactant causes an emulsion to form, which is broken by "salting out" with sodium chloride. After the phases have separated, the isooctane is filtered to deposit the chrysotile on a Nuclepore filter. Then, the filtered deposit is coated with vacuum-evaporated carbon. The carbon coating serves to make the preparation highly reflective and also increases the apparent fiber diameters to bring more fibers into the detectable range of light microscopy.

Light microscopic analysis is carried out at 500X magnification using incident bright-field (vertical) illumination to count fiber segments equal in length to the distance (12.5 μm) between concentric circles in an eyepiece reticle. The counts expressed as total fiber length are then translated into micrograms of chrysotile per filter deposit by reference to a standard curve that relates total fiber length to mass of chrysotile per filter. Finally, the chrysotile mass (μg) per liter of water sample is calculated from the aliquot taken, assuming 75 percent recovery.

The following detailed procedure presents step-by-step the instructions for carrying out TPLS coupled with light microscopic analysis.

Step 1: Shake the bottle of water sample with a mechanical shaker (Figure A-1) for approximately 5 minutes at ≈180 cycles per minute to create a uniform particulate suspension.

Step 2: Take an aliquot either directly from the water sample, or, alternatively, filter an aliquot onto a Metricel DM450 filter.
Alternative 2A: Take a 75-ml aliquot directly from the uniform water sample. Adjust the pH to 3.5 ±0.1 with 0.1 N HCl, add 25 ml of 0.01M MO-70, heat to 140°F

while stirring, and allow to cool to room temperature. If acid additions overshoot the correct pH value, correct by adding 0.1 N NaOH. Heat at least to 140°F; heating as high as the boiling point has no adverse effect except to extend the time required for cooling. Cooling may be accomplished by refrigeration or in an ice bath. Also, other than 75-ml aliquots may be taken; then the amount of surfactant added must be adjusted to maintain the 2.5×10^{-3} M concentration, and the amount of isooctane must be 1/3 of the total liquid volume.

Alternative 2B: Filter a relatively large aliquot (1 liter or more) of water sample to deposit the particulate on 47-mm Metricel DM450 (0.45 μm pore size) filter(s) (change filters if necessary as they become loaded to the degree that the water flow stops). Before the filter deposits have dried, ultrasonically remove the deposited particulate from the filter(s) in a beaker containing 75 ml of water to which 25 ml of 0.01 M MO-70 was added to make a total volume of 100 ml. Adjust the pH to 3.5 ±0.1 with 0.1 N HCl, heat to 140°F, and allow to cool to room temperature.

Step 3: Combine the water phase from Step 2A or 2B with 50 ml of isooctane in a 250-ml separatory funnel equipped with a Teflon stopcock.

Step 4: Attach the separatory funnel to a mechanical shaker and shake at ≈150 ±25 cycles per minute for 5 minutes (Figure A-2).

Step 5: Add 10 ml of 10% NaCl to break the emulsion (Figure A-3). Shake lightly to distribute the NaCl in the emulsion. Occasionally the isooctane layer will form under the aqueous layer. Further gentle shaking will eliminate this condition.

Step 6: Remove the separatory funnel stopper during separation of the two liquid layers. Drain off the water phase through the stopcock (Figure A-4). Allow time for the water droplets to settle and drain them off before pouring the isooctane from the top of the separatory funnel into the 25-mm Millipore filtering apparatus (Figure A-5). It is extremely important to take care that no water is filtered along with the isooctane. The isooctane is filtered through a 0.2-μm-pore-size, 25-mm Nuclepore filter, shiny side up, backed with an 8.0-μm-pore-size, 25-mm Millipore filter to obtain a uniform chrysotile distribution.

Step 7: Deposit the separated chrysotile onto the Nuclepore filter by aspirating the isooctane through the filter.

Step 8: Combine the same water phase again with 50 ml of isooctane in the separatory funnel and repeat the extraction procedure without adding additional sodium chloride, and deposit the extracted chrysotile on the same Nuclepore filter. Repeat the extraction procedure three more times to make a total of five extractions from the same water aliquot deposited on the same Nuclepore filter.

Step 9: Remove the Nuclepore filter, allow it to air dry and tape it by the edges, deposit side up, so that it lies flat on a 3 x 1-inch labeled microscope slide (Figure A-6). Double sided adhesive tape may be used to hold the filter flat for microscopic examination.

Step 10: Evaporate carbon onto the surface of filter bearing the deposit. (Evaporate 10.0 millimeters by 1.0 mm graphite rod at a distance of 20 cm at normal incidence.)

Step 11: Use vertical (incident bright-field) illumination to view the carbon-coated filter at ≈500X magnification with a light microscope equipped with an eyepiece reticle having

10 concentric circles (Figure A-7). In the eyepiece reticle, the radius of the center circle is equal to the distance between adjacent concentric circles.

Step 12: Position the filter beneath the objective lens so that a traverse may be made across the full diameter of the particulate deposit.

Step 13: Determine the smallest detectable fiber diameter. One count by the light microscopic method is registered for a fiber with the minimum detectable diameter and a segment length equal to the distance between concentric circles. At 500X, this distance is 12.5 μm in the Leitz Orthoplan microscope used at Battelle. For other microscope setups the field delineated by the concentric circles must be calibrated using a stage micrometer.

Step 14: Perform fiber segment counts on fibers that fall within the outside concentric circle as the major diameter of the filtered deposit is traversed. As counts are made, roughly estimate the number of minimum fiber diameters encountered in fiber bundles and adjust the fiber segment count accordingly.

Step 15: Translate fiber segment counts into total fiber length by multiplying the number of counts times the calibrated distance between adjacent concentric circles. Refer the total fiber length to the standard curve (Figure A-8), which relates total fiber length to chrysotile mass (μg) per filter.

Step 16: Calculate the chrysotile concentration (μg) per liter from the mass per filter obtained from the standard curve as follows:

$$\mu g/\text{filter} \times \frac{100}{75} \times \frac{1000\ \text{ml}}{\text{ml of water aliquot}} = \mu g/\text{liter}$$

This calculation is based on 75% recovery. When the outside circle of eyepiece reticle delineates a field 250 μm in diameter, and the major diameter of the deposit is 16 mm, one 250-μm-diameter field area is 1/82 of the area of the path 250 μm wide and 16 mm long across the filter. Because the standard curve was derived using this path area ($\approx$4.0 mm^2), areas measured with other microscope conditions must be determined and compensations made for differences by dividing the total path area by the area over which measurements were made and multiplying this ratio times the number of fiber counts times the calibrated distance between concentric circles.

When the radius of the smallest circle in a set of 10 circles is equal to the spacing between adjacent circles, the following area percentages may be used in calculations to normalize fiber length data obtained by counting less area than that within the outermost circle.

Circle No. 10	100%
Circle No. 9	81%
Circle No. 8	64%
Circle No. 7	49%
Circle No. 6	36%
Circle No. 5	25%
Circle No. 4	16%
Circle No. 3	9%
Circle No. 2	4%
Circle No. 1	1%

Making Up Solutions

It is imperative that all solutions be filtered before use. It is suggested that solutions be filtered through 0.2-μm-pore-size, 47-mm-diameter Millipore filters.

MO-70 (Dioctyl Sodium Sulfosuccinate)

MO-70 is obtainable from Mona Industries, Inc., Paterson, New Jersey 07524, in 70 percent aqueous solution. To make up the 0.01M stock solution, weigh out 6.34 ±0.02 g. Heat the solution to 140-150°F to obtain a uniform solution while stirring. Dilute to 1 liter with deionized water, and filter. (Shelf life – 1 month).

0.1 N HCl and 0.1 N NaOH

Purchase vial of Dilut-It (G. T. Baker Chemical Co.) for making up 0.1 N HCl or 0.1 N NaOH. Dilute the contents of the plastic ampoule to 1 liter with deionized water, and filter through 0.2-μm-pore-size, 47-mm Millepore filter.

10% NaCl

Add 90 ml of deionized water to 10g of reagent grade NaCl and filter.

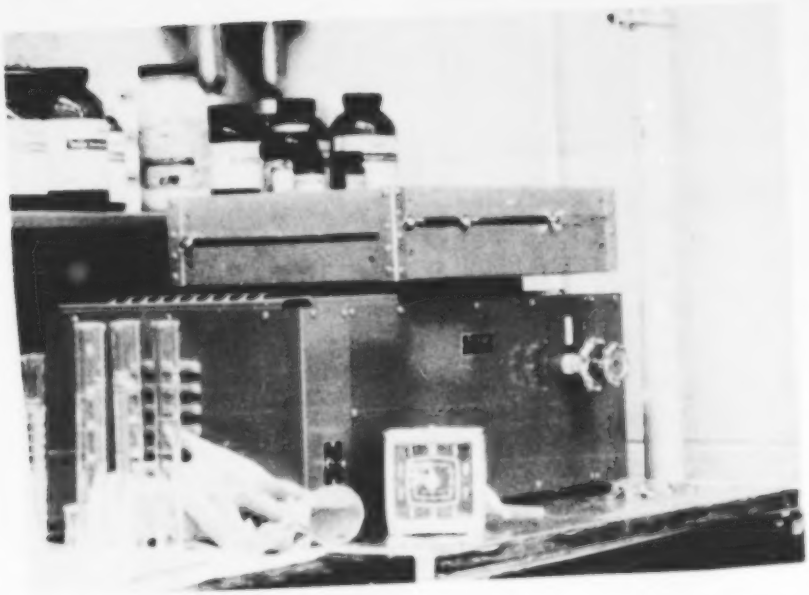

Figure A-1. The mechanical shaker.

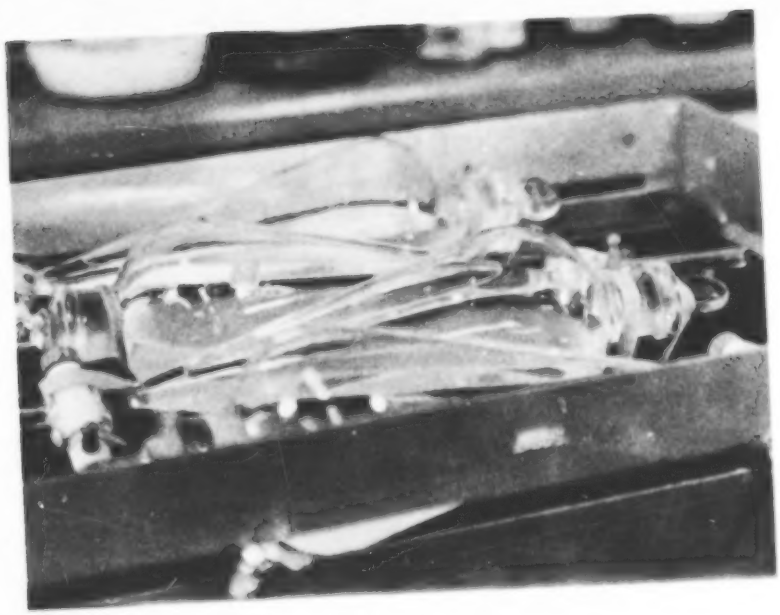

Figure A-2. Separatory funnels attached to mechanical shaker with rubber bands.

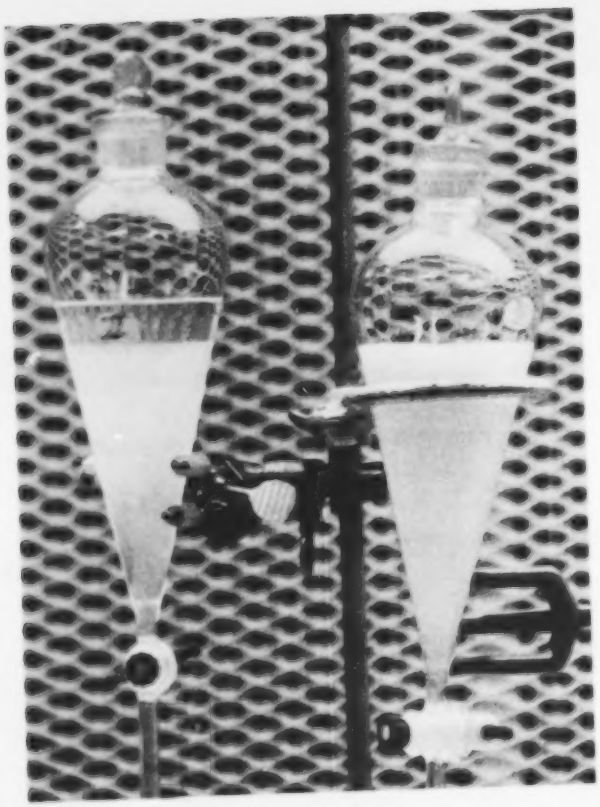

Figure A-3. Before (right) and after (left) breaking the emulsion by "salting out" with NaCl.

Figure A-4. Draining off the water phase.

Figure A-5. Filtering the isooctane.

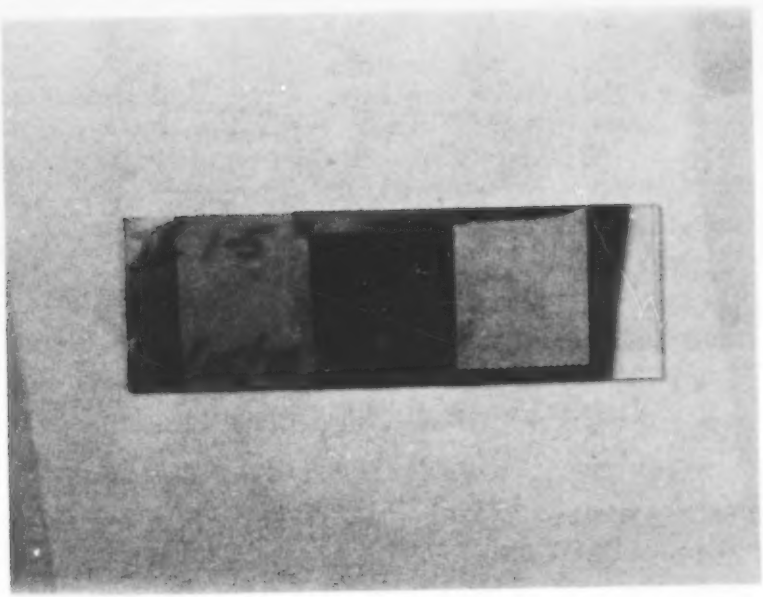

Figure A-6. A TPLS specimen prepared for light microscopic analysis. The filtered TPLS fraction was taped to a microscope slide and coated with vapor-deposited carbon in a vacuum evaporator.

Figure A-7. Concentric circles in eyepiece reticle superimposed on image of TPLS fraction from ORF water sample.

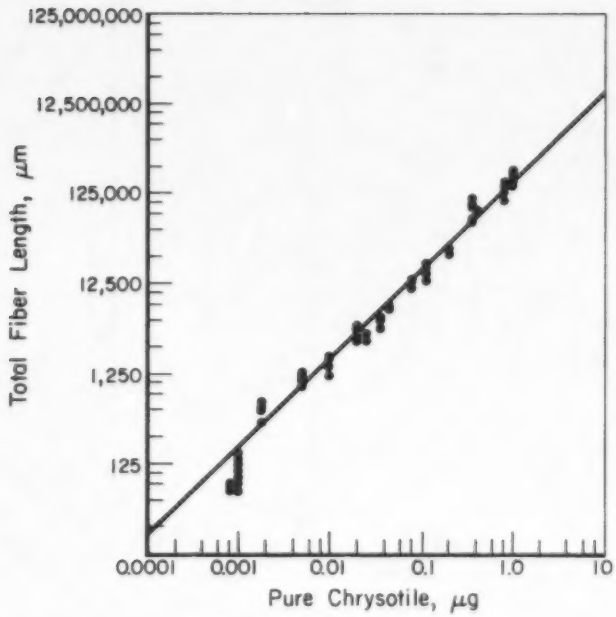

Figure A-8. Standard curve which relates light microscope measurement of total fiber length to mass (μg) of chrysotile per filter.

APPENDIX B

INSTRUCTIONS FOR CARRYING OUT TWO PHASE LIQUID SEPARATION (TPLS) COUPLED WITH SPOT TEST DETECTION OF CHRYSOTILE IN WATER SAMPLES

The rapid analytical method alternative to two-phase liquid separation – light microscopic detection procedure is the TPLS – spot test procedure. Although spot test detection is less sensitive than light microscopic detection (0.1 μg versus 0.001 μg), spot testing is more rapid, and its sensitivity is adequate for some monitoring situations. Spot testing also has the advantage of being routine, rapid, requiring no equipment as expensive as a light microscope, and very little training. After separation of the chrysotile is accomplished by TPLS, spot test detection can be run in 10 minutes.

The only equipment needed are a 25-mm Millipore filtering apparatus, a special reservoir with a 3-mm hole for the filter apparatus, a heat lamp, a wash bottle, spot test chemicals, and a spraying device such as is used in thin layer chromatography. The materials required are 25-mm-diameter, 0.45-μm-pore-size Millipore filters, spot-test chemicals (potassium periodate and tetrabase), and white blotters.

A technician who has no formal scientific training can be taught to run the spot test detection procedure in less than 1 hour. Semiquantitative results can readily be evaluated with a little practice to determine whether there are changes in chrysotile concentration within the range of detection of the method.

The chrysotile extraction procedure is the same as that recommended prior to light microscopic detection; however, a Millipore filter is used in the spot-test technique rather than a Nuclepore filter as is used for the light microscopic detection.

The selective transfer of chrysotile in the TPLS procedure occurs when the isooctane and water are shaken together in a separatory funnel after the chryostile surface is rendered hydrophobic through a surface reaction with the anionic surfactant MO-70, dioctyl sodium sulfosuccinate. The presence of the surfactant causes an emulsion to form, which is broken by "salting out" with sodium chloride. After the phases have separated, the isooctane is filtered to deposit the chrysotile on a Millipore filter. The deposit is confined to a 4-mm-diameter filter area to enhance spot test detection.

Spot test detection involves first the reaction of manganous ion with chrysotile extracted from a water sample followed by the detection of the reacted manganese by a spot test reaction which is catalyzed by manganese. The manganese catalyzes the reaction between potassium periodate and tetrabase [4, 4′ Methylenebis (N, N-dimethylaniline)] to yield a bright blue

reaction product. The depth of color developed is indicative of the quantity of manganese present in the chrysotile structure.

The following detailed procedure presents step-by-step instructions for carrying out TPLS coupled with chrysotile detection by the catalytic periodate-tetrabase spot test.

Step 1: Shake the bottle of water sample with a mechanical shaker (Figure A-1) for approximately 5 minutes at ≈180 cycles per minute to create a uniform particulate suspension.

Step 2: Take an aliquot either directly from the water sample, or, alternatively, filter an aliquot onto a Metricel DM450 filter.

Alternative 2A: Take a 75-ml aliquot directly from the uniform water sample. Adjust the pH to 3.5 ±0.1 with 0.1 N HCl, add 25 ml of 0.01M MO-70, heat to 140°F while stirring and allow to cool to room temperature. If acid additions overshoot the correct pH value, adjust by adding 0.1 N NaOH. Heat at least to 140°F; heating as high as the boiling point has no adverse effect except to extend the time required for cooling. Cooling may be accomplished by refrigeration or an ice bath. Also, other than 75-ml aliquots may be taken; then the amount of surfactant added must be adjusted to maintain the 2.5×10^{-3} M concentration, and the amount of isooctane must be 1/3 of the total liquid volume.

Alternative 2B: Filter a relatively large aliquot (1 liter or more) of water sample to deposit the particulate on 47-mm Metricel DM450 (0.45-μm-pore-size) filter(s) (change filters if necessary as they become loaded to the degree that the water flow stops). Before the filter deposits have dried, ultrasonically remove the deposited particulate from the filter(s) in a beaker containing 75 ml of water to which 25 ml of 0.01 M MO-70 was added to make a total volume of 100 ml. Adjust the pH to 3.5 ±0.1 with 0.1 n HCl or 0.1 n NaOH, heat to 140°F, and allow to cool to room temperature.

Step 3: Combine the water phase from Step 2A or 2B with 50 ml of isooctane in a 250-ml separatory funnel equipped with a Teflon stopcock.

Step 4: Attach the separatory funnel to a mechanical shaker and shake at ≈150 ±25 cycles per minute for 5 minutes (Figure A-2).

Step 5: Add 10 ml of 10% NaCl to break the emulsion (Figure A-3). Shake lightly to distribute the NaCl in the emulsion. Occasionally the isooctane layer will form under the aqueous layer. Further gentle shaking will eliminate this condition.

Step 6: Remove the separatory funnel stopper during separation of the two liquid layers. Drain off the water phase through the stopcock (Figure A-4). Allow time for the water droplets to settle and drain them off before pouring the isooctane from the top of the separatory funnel into the 25-mm Millipore filtering apparatus (Figure A-5). It is extremely important to take care that no water is filtered along with the isooctane.

A special filter reservoir is used to concentrate the chrysotile into a small area (Figure B-1). [The special reservoir, used as a substitute for the standard reservoir, is made with a 3-mm hole to concentrate the asbestos in a 4-mm-diameter filter area (Figure B-1). A glass blower can readily make this special part.]

Step 7: Aspirate the isooctane through a 25-mm, 0.45-μm, Millipore filter to deposit the extracted chrysotile.

Step 8: Combine the same water phase again with 50 ml of isooctane in the separatory funnel and repeat the extraction procedure without adding additional sodium chloride, and deposit the extracted chrysotile on the same Nuclepore filter. Repeat the extraction procedure three more times to make a total of five extractions from the same water aliquot deposited on the same Millipore filter.

Step 9: Remove the filter, air dry and store in a labeled plastic petri dish.

Step 10: Place the filter, deposit side up on the frit of a 25-mm Millipore filtering apparatus and place a blank Millipore filter on top of the filter bearing the chrysotile deposit. Clamp the standard Millipore reservoir (16-mm-hole) in position.

Step 11: Pour water into the reservoir and adjust the aspiration rate to 15 ml per minute.

Step 12: Aspirate 15 ml of 25% manganous sulfate (25 g $MnSO_4 \cdot H_2O$/100 ml solution) through the filter.

Step 13: Wash five times aspirating each washing completely through the filter before adding the next washing.

Step 14: Increase the aspiration rate to hold the filters to the frit and remove the filter reservoir.

Step 15: Separate the filters and discard the top filter.

Step 16: Place the bottom filter deposit side up on a white blotter and dry under the heat lamp at 145°F for 1 minute.

Step 17: Attach the filter deposit side up by its edges with cellophane tape to the white blotter.

Step 18: Use a spraying device (Figure B-2) to spray the filter two times lightly with the spot test working solution which is made up by mixing equal (5 ml) quantities of the following Solutions A and B:
Solution A: Saturated solution of potassium periodate in 2N acetic acid (11.5 ml glacial acetic diluted to 100 ml with water).
Solution B: 1.0 g of tetrabase dissolved in 100 ml of 95 percent ethyl alcohol.

Step 19: Place the filter under the heat lamp at 145°F until the color of the spot does not change, or if no spot becomes visible, until the filter background turns a light blue color.

Step 20: Compare the color of the spot to colors developed in similar preparations made from known chrysotile concentrations. The latter may be recorded on 35-mm color transparencies that can be viewed to make comparisons to unknowns. Color prints were not found satisfactory for this purpose.

A color Xerox copy of a spot test result obtained on an 0.8 μg chrysotile standard is shown in Figure B-3.

Note: Filter all solutions through 0.45-μm-pore-size Millipore filters before use.

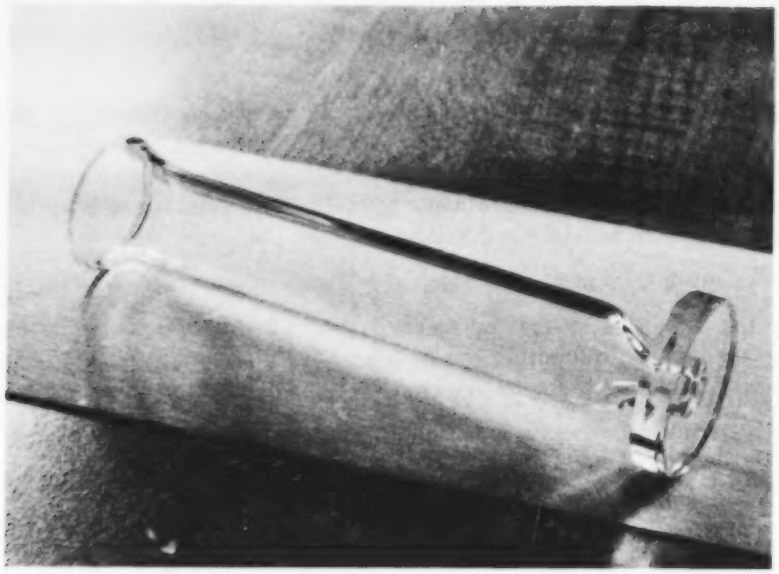

Figure B-1. Special reservoir with 3-mm-diameter hole.

Figure B-2. Spraying device with vial containing spot test working solution.

Figure B-3. A typical spot test result obtained on an 0.8-µg chrysotile standard.